

FLEXNER (S.)

✓

A STUDY OF THE BACILLUS (LEPTOTHRIX ?)
PYOGENES FILIFORMIS (NOV. SPEC.)
AND OF ITS PATHOGENIC ACTION

BY

SIMON FLEXNER, M. D.



FROM
THE JOURNAL OF EXPERIMENTAL MEDICINE
VOL. I, No. 2, 1896

✓

A STUDY OF THE BACILLUS (LEPTOTHRIX?) PYOGENES FILIFORMIS (NOV. SPEC.) AND OF ITS PATHOGENIC ACTION.

BY SIMON FLEXNER, M. D.

PLATES IX-X.

(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

TABLE OF CONTENTS.

History, p. 211—Description of the organism, p. 214—Experiments upon animals, p. 218—
The organism as obtained from the experimental cases, p. 231—Phagocytosis, p. 233
—Cultivation of the organism, p. 234—The histology of the affected organs, p. 241
—Résumé and general considerations, p. 265—Review of the literature, p. 275.

HISTORY.

A preliminary communication concerning this micro-organism appeared in the *Bulletin of the Johns Hopkins Hospital* for November-December, 1895. The source of the microorganism was one of the laboratory rabbits, of which the following is a brief history:

On March 13, 1895, a large healthy rabbit of the stock of the laboratory gave birth to a litter of young; five days later she was found dead in her cage. The young of this animal died a day or two preceding the death of the mother. The autopsy was performed in the afternoon of the 18th.

The animal was well nourished, and there was no evidence that death had been caused by violence. The mammary glands were still large and contained milk; the peritoneal cavity was smooth and glistening throughout and did not contain an excess of fluid. Upon examination of the abdominal viscera, however, the condition of the uterus arrested at-



tion. It was several times larger than normal, although much smaller than the uterus of the rabbit at term, and presented a series of dilatations and constrictions which, except for their irregular distribution and peculiar translucency, might have been mistaken for a condition of pregnancy. This possibility was, of course, not to be considered, and, indeed, the dilated and semi-transparent pouches gave the impression of being empty. The serosa covering these dilatations was injected, the larger vessels being very prominent and turgidly filled with blood, while the intervening tissue presented a rosy hue. Both cornua of the uterus were similarly affected. Nothing abnormal was observed in connection with the ovaries. The pallor of the interior of the uterus contrasted with the injection of the serous coat. Upon gently stroking the mucosa with a knife a thick, opaque material could be removed, which appeared to be only lightly adherent to the surface of the membrane. It was to the presence of this exudate, extending from the vagina throughout the uterus, that the pallor and opacity of the mucous membrane were due.

In the dilatations before mentioned the mucous membrane was of extreme tenuity, and here the layer of exudate was thinner than elsewhere. The dilatations were apparently produced by the accumulation of gas. After the removal of the exudate the underlying mucosa was found congested.

The pleural cavities contained an excess of fluid of a haemoglobin-red colour. The serosa itself was covered with a thick, shaggy layer of fibrin-like material. Both the parietal and visceral layers were covered with this material, which was thickest over the diaphragm. The lungs were in part voluminous and in part collapsed, the expanded portions being of firm consistence and apparently consolidated. The whole of the posterior lobe (caudal) was solidified, and the corresponding bronchi contained firm opaque plugs.

The pericardial sac contained a small amount of red fluid between layers of a shaggy exudate similar to that covering the pleuræ.

From the appearance described there could be little question that the death of the animal was caused by an acute pleuritis, pericarditis, and pneumonia, associated with acute endometritis, the presumption being that the causative infectious agent entered through the genital tract. It was thought that the infection had taken place at the time of or soon after parturition.

The bacteriological examination of the exudate contained within the genital tract as well as in the cavities of the thorax confirmed the supposition of infection, and revealed a micro-organism of striking and peculiar morphology. The same organisms were present in the two situations mentioned, although they were more abundant in the uterine cavity.

The discovery of large numbers of micro-organisms in cover-glass preparations led to the preparation of cultures from the different inflammatory exudates. The peculiar morphology of the bacilli to be described awakened the suspicion that it might be difficult, or even impossible, to cultivate them in the usual way and upon the ordinary culture media. Two methods of procedure were therefore employed. In the first place, transplantation of the exudate from the uterus, pleura, and pericardium was made into several kinds of culture media, and these were kept under aerobic and anaerobic conditions. The media employed were as follows:

1, Nutrient gelatin; 2, agar-agar; 3, bouillon; 4, milk; 5, potato; 6, Loeffler's blood serum (from bullock's blood); 7, the same prepared with human blood serum; and 8, the mixture of blood serum, urine, and agar (*Steinschneider*).

Anaerobiosis was obtained by the use of Buchner's jars and by the substitution of hydrogen for air. These culture tubes remained in the thermostat at 37° C. for many days without manifesting any growth whatever. Subsequently attempts were made to cultivate the organism by the use of the coagulated blood and blood serum of the rabbit, but without success. Sterile blood was collected from the femoral artery of the rabbit in Nuttall's bulbs, and after twenty-four hours the separated serum was pipetted off into test tubes. Some of these tubes were heated to 60° C. for thirty minutes, to destroy the bactericidal property of the serum, while the remainder were permitted to remain "active." Bits of the pleural exudate obtained from subsequent cases were transplanted to the blood serum and upon the clot which remained in the bulb after the separation of the serum. Placed in the thermostat, no growth could be detected even after the lapse of two or more weeks. The replacement of the air in the serum tubes by hydrogen did not alter the result. The use of more concentrated media was also tried. Bouillon and agar-agar containing five per cent. of peptone, the other constituents remaining unaltered, were equally unsuccessful, as were also media containing other reducing substances than sugar—namely, formate of sodium (one per cent).

A small bit of the solid exudate obtained from the pleural cavity of the first animal was broken up in sterile salt solution and injected, after a small skin incision had been made, into the right pleural cavity of a second rabbit. The inoculation was positive in its results.

DESCRIPTION OF THE ORGANISM.

Cover-glass preparations made from the exudate in the uterus show a surprisingly large number of bacilli, which formed a felted network upon the surface. In the interstices between the bacteria a moderate number of mononuclear cells are contained. Cells with more irregular forms of nuclei are more exceptional. The greater part of the solid exudate in this situation is composed of the bacilli; there is considerable variation in the size, and some variation in the appearances of the latter. The size varies from 1.4μ to 154μ , which represent the extremes. The majority of the longer forms vary between 56μ and 70μ . The average width of the bacilli is from 0.5μ to 0.7μ . Notwithstanding this great difference in size, the peculiar appearances which we shall describe leave no doubt that only one species is present. The forms of the longer ones, which sometimes reach nearly across the field of the microscope (oil immersion one-twelfth, ocular No. 3) are not straight, but curved. The curves are delicate, long, and sinuous. For the most part the bacilli do not lie in parallel rows. Very few of them in a given field are of the same length or originate from the same point. They frequently cross one another, are, perhaps, curved upon themselves so as to give a looped appearance, and through these differences in direction and form bring about the felting before mentioned. This peculiarity of arrangement, it is believed, is not due to mechanical changes caused by spreading the exudate upon the cover slip, for the lack of uniformity in the thick and undisturbed portions of the preparation is more marked than in the thinner ones, where a rearrangement may have been produced.

Only a very few of the bacilli stain uniformly throughout. For the most part they stain irregularly, and indeed this irregularity of staining is one of their most striking characteristics. It is present

in the small forms as well as in the longest and thus confirms the belief in the similarity of nature of the organisms. The bacilli are composed of longer and shorter strands of protoplasm, which, as compared with the brightly staining points, take on a very faint colour with aniline dyes (Plate IX, Fig. 1). This material forms the matrix in which are placed at regular intervals deeply stained points of chromatin. These deeply staining points, while varying somewhat in size in different bacilli, are, in a given organism, about equidistant from one another. They are cylindrical rather than globular, generally longer than the width of the protoplasmic material in which they lie. The smallest forms contain usually two such brightly staining points, although some contain only one. The chromatin points are never at the ends of the latter, the extremities of the bacilli being composed of the lightly staining protoplasmic envelope. Although the smaller forms may contain only one, they more commonly possess two or three specks, while the longest ones often contain in the neighbourhood of one hundred. The ends of the bacilli are rounded in the large as well as in the small forms, and the former show no evidence of transverse segmentation, which might indicate that they are composed of chains of the smaller forms. There is no evidence whatever that in the uterus the bacteria have been taken up by cells, nor were any appearances observed that indicated a lateral branching.

The bacilli as they appear in the cover slips prepared from the pleura of this case correspond in all essential particulars with those present in the uterus. A few minor points should, however, be noted. There are fewer of the short forms as compared to those in the uterus, although the total number of bacilli is much less than in the latter. This leaves a larger proportion of the longer ones, and indeed this is a striking feature of the exudate in the pleural cavity. The number of solid forms is also greater in this situation, a fact which may be due in part to the albuminous fluid in which they are contained which is rather difficult of decolourization. In the uterus the thickness of the bacilli is distinctive, but in the pleura some variation in this respect is apparent. The protoplasmic strands

are all finer, wider, and the stained chromatin cylinders a little thicker than in the uterus. There is, too, some irregularity in the size, form, and separation of the latter. Altogether there is more variation among the forms in the pleural than in the uterine exudate. The cells which are contained in the exudate are composed of polymorpho-nuclear leucocytes, cells with single oval nuclei and abundance of cell protoplasm and red blood-corpuses, the last in considerable numbers and sufficient to give to the pleural fluid its red colour. Cells with polymorphous nuclei predominate.

Staining.—The bacilli stain readily in all the aniline dyes. Carbolic-fuchsin and aniline—oil gentian violet (Stirling) stain them very quickly. The irregularity of staining comes out best after treatment of the stained exudate by a 1 to 1,000 watery solution of glacial acetic acid. Without this treatment this feature is much less evident. Stronger bleaching agents (one-per-cent sulphuric acid, Neisser) produce rapid and complete decolourization of the organisms; the use of a solution of Bismarck brown following upon methylene blue, as recommended by Ernst, deprives the chromatic particles of the latter stain. The immersion of the heated cover slips in Delafield's haematoxylin for several hours gives a sharp stain of purplish-black colour to the dots and a pale-violet tint to the matrix in which they lie. On the other hand, the bacilli are decolourized upon cover slips both by Gram and by Weigert's methods, and they are not stained by iodine. The fluid which can be collected from the pleural and pericardial cavities contains the bacilli in smaller number than the solid exudate. If a drop of this unheated fluid containing the living bacilli be mixed with a small drop of carbolic-fuchsin or gentian violet and covered with a slip, the chromatin points can be observed to take the stain, leaving the protoplasmic envelope slightly tinted only. The chromatin must therefore be considered to be pre-formed in the manner described within the rods. As C. Fraenkel was enabled by a similar method to discover the branching of the bacillus diphtheriae, this phenomenon was looked for, but no indication of it was observed.

Motility.—The bacilli are non-motile. Examination of the pleural fluid at once after the death of the animal by means of hanging drops showed entire absence of motility. Bits of the exudate which were broken up in salt solution and examined in this way gave the same result.

Thermal Death Point.—Capillary tubes were filled from the fluid exudate contained within the pleural cavity of an inoculated animal which showed fair numbers of typical bacilli. These tubes were exposed to a temperature of 55° C. for five minutes, and, the contents of several having been mixed, they were introduced into the pleural cavity of another rabbit. No effects resulted. Control animals inoculated directly from the pleural fluid which had not been subjected to this temperature succumbed at the end of a few days. It must therefore be concluded that at the temperature of 55° the bacilli are killed in five minutes, or at least are deprived of all virulence.

Question of Spore Formation and Vitality in Exudates.—The low thermal death point of the organisms indicated that, within the body at least and a short time after death, spores were not formed. The short length of time of their survival outside of the body is indicated by the following experiments, and these would further tend to show that spores are not formed at all. On May 2d a bit of the solid exudate derived from the pleura of an animal which had been autopsied the same day was transferred with the usual precautions to avoid contamination to a recently sterilized test tube. Over the cotton plug a rubber cap was fitted and the tube was put aside in a cupboard exposed to diffuse daylight. On June 18th the tube was examined. Cover-slip preparations prepared from the exudate which was still moist showed typical bacilli, which, however, stained faintly, and besides these the inflammatory cells which now stained very poorly indeed. No other organisms were found, nor were bright unstained sporelike bodies observed. This exudate was broken up in sterile bouillon and injected into the pleural cavity of a half-grown rabbit without any result whatever. The effect of drying upon the bacilli is shown by the following experiment:

8. *The Bacillus Pyogenes Filiformis and its Pathogenic Action*

On May 26th several bits of the solid exudate derived from the pleural cavity were placed in recently sterilized Petri dishes and slowly dried in the thermostat at 37° C. They remained in the thermostat for forty-eight hours, at the end of which time they were removed and for a short period were exposed to diffuse daylight. Portions of the dried exudate were then powdered and suspended in sterile bouillon. Cover slips prepared from this suspension showed well-staining bacilli. The inoculation of the suspension into the pleural cavity was entirely without effect upon rabbits. The conclusions to be drawn from these experiments are, first, that neither the stained nor the unstained areas in the bacilli are to be regarded as spores; second, that it is very probable that spores are not formed without the body, at least not under the usual conditions of their formation; and, finally, that the vitality of the organism in the outer world is not great.

EXPERIMENTS UPON ANIMALS.

Intrapleural Inoculations.—The failure to cultivate this organism necessitated its transference from animal to animal in order that it might be kept alive sufficiently long to permit the study of its properties. It has already been stated that the inoculation from the pleural exudate of the original rabbit into the pleural cavity of a second one was followed by the death of the latter animal. The conditions which were found in the thorax of the experimental animal were quite indistinguishable from those observed in the original case. Intrapleural inoculations were carried through a series of sixteen rabbits. These covered a period extending from the 19th of March to the 4th of June. Among the rabbits used for these experiments were large full-grown, as well as smaller, one-third- to one-half-grown animals. The amount of material inoculated varied considerably. In all instances the inoculations were made into the right pleural cavity, the precaution always being observed to make a slight skin incision before introducing the needle of the hypodermic syringe into the cavity. As much care as possible was taken not to injure the adjacent lung. The material which was inoculated was

derived in most cases from the pleura of previously inoculated animals, the haemorrhagic fluid being the part generally chosen. In exceptional cases, however, material containing bacilli derived from the infected subcutaneous tissues, inflamed peritonæum, or visceral abscesses was, after suspension in sterilized fluids, used for the inoculations.

The first of this series of experiments was made on March 19th and the last on June 4th. The period of incubation varied considerably, the longest time observed being five days and the shortest twelve hours. It is, however, necessary to state that the longest incubation was in connection with a large rabbit to which a small quantity of the exudate from the first case had been given, and the shortest a small animal which had received relatively a large amount of the infectious material. There would appear to have been an increase in virulence developed by the successive transference of the bacilli from animal to animal.

TABLE I.

	Source of infectious material.	Amount inoculated.	Size of rabbit.	Date of inoculation.	Date of death.
1	Uterus of original rabbit.	Bit of the exudate suspended in bouillon.	Large.	March 19, 3 P. M.	March 24, night.
2	Pleural exudate <i>idem.</i>	Bit of the exudate.	One-third grown.	<i>Idem.</i>	March 24, 9 A. M.
3	Pleural exudate of last.	Bit of the solid exudate.	One-third grown.	March 24, 3 P. M.	March 28, 5 P. M.
4	Pleural exudate.	0.25 c. c. of the haemorrhagic fluid.	One-half grown.	March 29, 10 A. M.	April 1, 4 P. M.
5	Pleural exudate.	Bit of the solid exudate.	Large.	April 11.	April 14.
6	Pleural exudate.	Small amount of the solid portion.	Large.	April 15, noon.	April 20, 11 P. M.
7	Pleural exudate.	0.25 c. c. of the fluid.	Large.	April 20.	April 23.
8	Edematous fluid from subcutaneous tissues.	0.2 c. c.	Large.	April 25, 3 P. M.	April 29, night.
9	Pleural exudate.	0.5 c. c. of the fluid.	Large.	April 29, 4 P. M.	May 1, night.
10	Pleural exudate.	0.5 c. c. of the fluid.	Nearly grown.	May 1, 3 P. M.	May 3, 2 P. M.
11	Peritoneal exudate.	A few drops of the fluid.	One-third grown.	May 11, 11 A. M.	May 14, 10 A. M.
12	Peritoneal exudate.	0.33 c. c. of the fluid.	One-third grown.	May 13, 3 P. M.	May 14, 3.30 P. M.
13	Pleural exudate.	0.1 c. c. of the fluid.	Large.	May 21.	May 24.
14	Liver abscess.	Suspension of bit of exudate.	Large.	June 1, 4 P. M.	June 4, night.
15	Pleural exudate.	1 c. c. of the fluid.	One-half grown.	June 4, 5 P. M.	June 5, night.

The table on the preceding page, which gives the dates of inoculation and death, the source of the bacilli, the relative sizes of the animals, and the amount of infectious material employed, is intended to bear upon this point.

The lesions which are produced by the intrapleural inoculation of this organism are so nearly uniform that a separate description of the cases appears hardly necessary. A typical case will be given, and then the more exceptional lesions will be spoken of specifically :

A large male rabbit was inoculated on the 25th of April at 3 P. M. with 0.2 cubic centimetre of the fluid derived from the pleural cavity of an animal autopsied on this date. On April 29th, at 8 A. M., it was found dead.

At the autopsy there was no reaction in the external tissues about the seat of inoculation. The right pleural cavity into which the inoculation had been made was covered with a shaggy, yellowish, pseudo-membranous exudate, forming a uniform covering over the visceral and parietal layers of the pleura, which averaged 1 to 2 millimetres in thickness. The greatest thickness occurred over the diaphragm, where the exudate reached 4 or 5 millimetres in depth. The right lung was compressed, dark red in colour, and completely atelectatic. No distinct pneumonia was visible. A few cubic centimetres of a red, distinctly haemorrhagic fluid occupied the dependent portions of the cavity. The left pleura did not contain a distinct pseudo-membrane, but it contained an increased quantity of fluid and the surface of the serosa was faintly opaque and covered by a distinctly glutinous material. An exudate similar to that covering the right pleura was present in the pericardium, both layers of which were covered with a fine shaggy, yellowish pseudo-membrane. An increased amount of a faintly reddish serum existed within this cavity. The mediastinal tissues were swollen, congested, and infiltrated with a gelatinous serum, containing bubbles of gas. The exudate on the serous surfaces was readily removed, exposing the congested and inflamed membranes beneath. The remaining viscera, with the exception of the liver, which showed smaller and larger points of yellow, opaque necrosis, were apparently normal.

The exceptional cases were, in the first place, those in which the animals succumbed in from twelve to twenty-four hours, in which instances the solid exudate in the pleural cavity, although much reduced in amount, was still present. The transuded fluid was never absent and it always presented the reddish colour previously mentioned. As early as twelve hours after the inoculation there was evidence of a great increase in the organisms introduced. In another instance, in addition to the typical lesions which occurred in the

pleura and pericardium, an abscess as large as a split pea was present in the wall of the left ventricle. It was opaque and firm and involved the entire thickness of the myocardium. In a further small number of cases the extension of the inflammatory process from the pleura could be followed into the peritoneal cavity, where it was localized upon the cephalic surface of the liver, corresponding to the part in contact with the surface of the diaphragm. The pseudo-membrane present here sometimes reached a size 3×3 centimetres in extent. It was thin and opaque, and after its removal, which was easily accomplished, the capsule of the liver appeared thickened and opaque. An exceptional instance of extension to the peritoneum with the production of a general peritonitis is the following:

A small one-third grown rabbit received into its pleural cavity on May 13th, at 3 P. M., 0.33 cubic centimetre of the peritoneal fluid from a case of peritoneal infection, which was autopsied the same day. It was dead at 3.30 P. M. May 14th.

The right pleura, into which the inoculation had been made, presented a pinkish colour and contained a small excess of fluid and a few flakes of fibrin adherent to its surface. This fibrin was found upon examination to contain the typical micro-organisms. Neither the opposite pleura nor the pericardium was involved. The peritoneal cavity contained an excess of fluid. The intestines were moderately distended and small flakes of a fibrinlike material were present on the omentum and the mesentery. Covering the right side of the liver there was a thick exudate. On section of the liver, this was seen to correspond with areas of smaller and larger abscess formation which occupied both the caudal and cephalic lobes of this side. The false membrane was continuous over the parts of the liver in which the abscesses were contained; it was scattered as small foci in other parts.

There can be, I think, little doubt that the peritonitis in this instance followed the extension of the liver abscesses to the capsule of the liver. Moreover, it illustrates the fact that such profound pathological lesions as these, associated with a great increase in the number of bacilli, can take place in so short a time as twenty-four hours.

In two instances, through some fault in the technique employed, the material containing the bacilli came into contact with the soft tissues over the thorax as well as with the pleural cavity. In one of these cases there was an inflammatory, oedematous exudate extending

over a considerable part of the thoracic wall and containing bubbles of gas. In the second the subcutaneous tissues and muscle were densely infiltrated with a thick, yellowish, firm material, which rendered the tissues hard and boardlike, the involved muscle appearing opaque and necrotic.

Pneumonia as a complication of the pleural cases occurred in several instances. It was present, it will be recalled, in the spontaneous case involving the caudal lobe of the right lung; in the experimental cases it was limited exclusively to the right side and usually involved less than an entire lobe. In one instance what appeared to have been an extension from the lung tissue to the bronchi was observed, and in this one the main bronchus proceeding to the affected lobe was plugged by a firm exudate.

The intrapleural inoculation of guinea-pigs was also followed by positive results. The following is a typical case:

A guinea-pig was inoculated into the pleural cavity at 4 P. M. on April 20th with 0.33 cubic centimetre of the pleural fluid derived from a rabbit dead the same day. The animal was observed to be ill on the morning of the 21st and was found dead on the 22d at 7 A. M.

The autopsy showed entire absence of reaction about the seat of inoculation. The right pleura was covered with a sticky, tolerably thick, opaque, greenish-white, slightly adherent pseudo-membrane. The lung was compressed, apparently free from consolidation, and an increased quantity of fluid was present in the pleural cavity. The exo-pericardium was covered with a thinner exudate than the lung, and its inner surface was injected, the sac itself containing turbid fluid. A thin layer of exudate covered the left pleura. The lung on this side was not compressed, although there was an increased amount of fluid in the pleural cavity. The liver showed several superficial small necroses. Cover slips prepared from the solid and fluid exudate exhibited typical bacilli, among which there were both polymorpha-nuclear and mononuclear cells. It was noticed in this case that a small number of the bacilli had been taken up by leucocytes. The long forms, which often exceeded the size of the pus corpuscles in which they were contained, were curved so as to accommodate themselves to the size of the phagocyte. The contained bacilli stained for the most part more feebly than those outside. In one instance a bacillus was seen half within and half without the corpuscle. The part within stained more feebly than the part without.

Intraperitoneal Inoculations.—In no instance did an animal survive intrapleural inoculation. It is interesting, therefore, to note

that inoculations into the peritonæum, although generally successful, failed in some cases and that the period of incubation was at times longer than in the pleural cases.

On April 20th a large rabbit received intraperitoneally 0.33 cubic centimetre of the pleural fluid from a rabbit dead that day. It was found dead on the morning of the 25th.

At the autopsy the tissues over the ventral (inferior) surface of the body were enormously swollen. The swelling affected particularly the subcutaneous tissues extending from the mid-portion of the thorax almost to the pubes and laterally to the axillary line. The infiltrated tissues were fully 1 centimetre in thickness; they were gelatinous and blood-stained. The muscles appeared to have escaped. In the superficial layers the subcutaneous tissue was intimately infiltrated with fluid, which manifested little tendency to escape, whereas in the deeper parts just above the muscles the tissues were swollen and necrotic. Gas bubbles were present both in the superficial oedematous tissue and in the deeper necrotic parts. Although the muscles just beneath the superficial fascia appeared to have escaped, the recti on both sides were found to have been converted into a yellowish necrotic material of almost stony hardness. The necrosis extended through the entire muscle and the subserous tissues, and, corresponding to the latter, an acute exudate covered the peritoneal coats. The peritonitis thus described involved the lower portion of the right lobe of the liver and that part of the cæcum which is situated immediately below the right lobe of the liver, where the exudate reached 1 millimetre in thickness.

Cover-slip preparations were made from the subcutaneous oedema, the necrotic muscle, the localized peritonitis, and from the general peritoneal cavity. The bacilli were present in all situations excepting the last.

The interpretation of this case is not entirely clear. It must at least be considered that the inoculation may have failed to reach the peritoneal cavity, that it entered only the subcutaneous tissues over the abdomen, and that subsequently an extension to the peritonæum took place from the necrotic abdominal muscles. While the reverse infection can not be positively excluded, it is, we think, hardly probable, particularly as we expect to show that the subcutaneous inoculations lead in themselves to extensive necrotic conditions of the soft tissues.

On May 8th a large rabbit received into the peritoneal cavity 0.33 cubic centimetre of the pleural fluid of a previous rabbit. The animal was dead in forty-eight hours.

The peritoneal cavity contained a considerable quantity of sticky fluid and large fibrinous flakes. The greatest accumulation both of fluid and

fibrin was in the pelvis, although there was a rich accumulation of lymph between the liver and the diaphragm. The serosa generally was injected and small bits of fibrin were attached to various parts of the visceral and parietal layers. The fluid portion of the exudate presented a distinctly glutinous character.

Cover-slip preparations showed many of the characteristic bacilli.

This case is an example of a pure peritoneal infection with rapid termination, while the following one is an example of long incubation :

On April 29th a large rabbit received 0.5 cubic centimetre of the pleural fluid of a previous animal. It was found dead on the 6th of May, having in the meantime lost some in weight.

About the point of inoculation there was some oedema of the subcutaneous tissues. The peritonæum did not show a uniform distribution of the inflammatory exudate. In the lower zone of the abdomen a small collection of sticky fluid existed, whereas small white masses were scattered at various places over the parietal and visceral peritonæum and the mesentery and omentum, the larger masses occurring between the loops of the intestine. The blood vessels of the serous membrane were injected. Between the diaphragm and the liver the pseudo-membrane was thickest. The under surface of the diaphragm appeared inflamed, both pleural cavities contained a sticky fluid, and the mediastinal tissues were infiltrated and oedematous.

Cover-slip preparations from the peritoneal cavity, from the pleural cavity, and from the mediastinal tissues showed the typical organisms.

The last two cases show that this organism, when introduced directly into the peritoneal cavity, is capable of setting up an acute, fatal peritonitis. It is interesting to note that in the last case an extension of the process—probably through the diaphragm, possibly through the mediastinal tissues—into the pleura occurred.

Intrauterine Inoculation.—A pregnant rabbit was laparotomized on May 11th, at 3.30 P. M., and received 0.33 cubic centimetre of a faintly turbid suspension, prepared from a fibrinous flake obtained from the peritoneal exudate, directly into one of the dilated segments of the pregnant uterus. The inoculated fluid had been examined by means of cover-slip preparations, and it was observed to contain very few of the micro-organisms. The wound in the abdominal walls was closed with silk sutures—first the peritonæum, then the skin, and the whole covered with a celloidin dressing. Strict antiseptic precautions were maintained throughout. The animal quickly recovered from the anaesthetic, but a hernia soon developed. Death occurred on May 13th, at 1.30 P. M.

At the autopsy the skin incision was found intact. There was no sup-

puration. A small knuckle of gut, covered with omentum, protruded through the lower angle of the wound and lay between the peritonæum and the subcutaneous tissues. The tissues covering the symphysis pubis and in the inguinal regions were emphysematous and oedematous. It may be mentioned that in this fluid no micro-organisms could be detected upon the cover slips. On removing the omentum over the protruded loop of intestine, small haemorrhages were observed beneath the serosa, covering some of which there was a deposit of yellowish fibrin. Other small haemorrhages occurred in the serosa of the large intestine. The peritoneal cavity itself contained an excess of bloody fluid, similar to that seen in previous cases in the pleural cavity. In the more dependent parts a thin, puslike exudate had collected. The segment of the uterus into which the injection was made was much congested, and was beset with small haemorrhages. The remaining segments of the right side (side of inoculation) were congested, while on the left side they had collapsed.

Upon opening the uterus, one foetus only was found, and this corresponded with the segment into which the inoculation had been made. It was suffused with blood, dark in colour, and covered with a grayish, thin exudate, which also covered the mucous membrane unoccupied by foetal structures. The left cornu of the uterus was more dilated, notwithstanding its collapsed condition, than the right. It contained a thin, opaque, sanguinolent fluid in considerable amount. What appeared to be remains of foetal structure were found, although, with the exception of the one foetus described, no others were present within the uterus.

The examination by means of cover slips of the fibrin and fluid in the peritoneal cavity, as well as the contents of both cornua of the uterus, gave typical bacilli, varying somewhat in numbers in different parts.

The effect of this inoculation was, undoubtedly, to cause an abortion in the animal, inasmuch as, at the time of the operation, the various dilated segments of the uterus were observed to contain foetuses. Notwithstanding precautions which were taken to avoid infection of the peritonæum, an acute peritonitis due to the bacillus pyogenes filiformis developed. In a previous instance an attempt had been made to inoculate directly into the pregnant uterus from the vagina, but without success. It was thought that by this means it might be possible to reproduce the precise chain of events which were supposed to have occurred in the original animal, but, as there is a great probability that the infectious material never entered the uterus at all, the failure of this experiment was not unlooked for. In the present instance of direct inoculation through the laparotomized peritonæum partial success only was achieved. The pleura

and pericardium, as well as all the extraperitoneal viscera, escaped infection.

Meningeal Inoculations.—On April 25th, at 3.30 P. M., a full-grown rabbit was anaesthetized, and, after trephining over the mid-portion of the skull, a bit of the exudate, derived from the subcutaneous tissues of another rabbit autopsied the same day, was introduced with antiseptic precautions beneath the dura mater. No immediate effect was produced by the operation. The animal died at 2 P. M. on April 25th, thus having lived twenty-two and a half hours.

Upon the removal of the celloidin dressing and the separation of the skin over the trephined opening, there was an escape of pus mixed with gas bubbles from the wound. Cover-slip preparations, prepared from this material, showed it to contain the introduced bacilli in pure culture. The tissues of the scalp over the side of the incision were oedematous over a considerable area, and contained the characteristic bacilli. The meninges covering the convexity of the brain were hyperæmic, and here and there ecchymotic. They were also somewhat oedematous. On the left side of the brain, about its mid-portion and 3 millimetres from the superior longitudinal fissure, an elevated excrescence 1.5 millimetre in extent was found. This corresponded with the trephined area and the seat of inoculation, and it was evident the inflammation had spread from this point.

Cover-slip preparations from the various portions of the cerebral meninges showed the typical organisms in varying numbers.

After hardening the brain in alcohol, an incision carried through the protuberance mentioned corresponded with a cavity in the cortical substance about the size of a split pea, the walls of which were smooth and which could readily have been made by the accumulation of a gas. A second rabbit, which had been inoculated in a manner similar to the last one, succumbed in about twelve hours. The lesions found at autopsy were similar to those already described, although they were perhaps less marked. œdema and gas formation were found in the tissues about the trephined opening; beneath the dura a small collection of pus corresponded to the site of the inoculation, and from this point the remainder of the meninges over the convexity were infiltrated with pus and serum. Only micro-organisms having the appearance of those introduced at the operation were present. In neither case was there any development of metastatic inflammatory processes in other parts of the body.

Subcutaneous Inoculation.—In connection with one of the cited cases, in which the inoculation was made into the peritonæum, it

will be recalled that there was considerable involvement of the subcutaneous tissues and muscles of the abdominal walls. That fatal results may follow purely subcutaneous inoculation is shown by the following case :

A large white rabbit was inoculated into the subcutaneous tissues and muscles of the right side of the thorax. Corresponding with these tissues there was present at the autopsy a very extensive opaque, yellowish-white, rather dry necrosis and exudation, involving the tissues from the skin nearly to the bone. The involved tissues occupied a large, irregular area, beginning near the middle line in front and extending over the right side of the thoracic wall nearly to a line drawn from the axilla to the crest of the ilium, and reaching 6 to 7 centimetres in length. Surrounding this sharply defined but rather irregularly outlined necrotic area there was a moderate gelatinous oedema with occasional dark-red haemorrhages. The involved muscle was firm, infiltrated, and showed an opaque, whitish necrosis.

Cover-slip preparations prepared from the necrotic tissue showed large numbers of the characteristic pleomorphic bacilli. The remaining viscera were free from localization of the organism.

Intravenous Inoculations.—Perhaps the most interesting, certainly the most widespread, results were obtained by the intravenous inoculations of the pleural fluid. The results were uniformly fatal, the animals succumbing in from two to four days after the inoculation. At autopsy, abscesses were present in the viscera. These were generally miliary in size, although at times they were larger and spreading. Preference was exhibited in reference to their localization, certain organs being entirely spared. The abscesses were never absent from the brain and heart muscle; they appeared occasionally in the liver, more rarely still in the voluntary muscles, never in the kidneys, spleen, or lungs.

On May 8th, at 10 A. M., a grown rabbit received 0.16 cubic centimetre of the pleural fluid into the ear vein. The animal died May 11th, at 10 A. M.

At the autopsy there was an increased amount of fluid of a sticky character in the peritoneal cavity which contained the characteristic organisms. On both surfaces of the peritonæum fibrin flakes were present. The liver was especially remarkable, as it was covered by a pseudo-membrane, which in part was thick and opaque. The membrane was present in greatest amount over the caudal lobe just above the stomach. Upon incision of the liver, it was found to be the seat of many abscess formations, the largest

occupying the caudal lobe mentioned. Except in the latter situation the abscesses were small, whereas in this lobe a large abscess, involving its entire thickness and almost its entire extent, existed. Apparently it was produced by the coalescence of many of the smaller abscesses. As these were frequently situated in this part of the liver as well as in others directly beneath the capsule, the extension of the inflammatory process to the peritonæum probably took place at these points. The heart muscle contained a few abscesses. A very few were present also in the substance of the brain. None occurred in the kidneys, spleen, lungs, or voluntary muscles.

The effects of the intravenous inoculations with respect to the point of localization of the bacilli were in part determined by the local conditions. For example, the inoculation into non-pregnant female animals was not followed by the reappearance of the micro-organisms in any of the structures of the genital tract, whereas in the case of pregnant animals the inoculations were followed by the localization of the organisms and the inflammatory process in the pregnant uterus. Again, the occurrence of previous disease—as, for example, psorospermosis in the liver of the rabbit—determined, apparently, the localization of the infectious material with the production of large and spreading abscesses in this viscus.

On April 29th, at 4.30 P. M., a pregnant animal received 0.5 cubic centimetre of the pleural fluid into the marginal ear vein. There were no immediate effects. On the morning of May 1st the rabbit was observed to be in convulsions. At 8.30, at which time I saw it, it was lying on its left side, with its spine curved and its head drawn back, until it touched the vertebral column. Slight movements or noise brought on severe convulsions. The latter usually began in the extremities, but rapidly extended over and involved the whole of the body. Placed on its right side, the animal soon returned to the left, although the convulsions made this difficult to accomplish. The eyes converged to the inside. The convulsions continued to occur from time to time during the day, the animal growing perceptibly weaker. There was subnormal temperature before death. Measured in the rectum at 2 P. M., the thermometer registered 32° C. The animal was found dead at 8 A. M. on May 2d. It was cold and stiff, and had evidently been dead some hours.

Autopsy.—The brain showed many abscesses. They averaged the size of a hemp seed, and were situated in both hemispheres and in the frontal, temporal, and sphenoidal regions. They were not absent from the base of the brain, although, apparently, they were most numerous in the cerebral cortex, a single one only having been discovered in the left lateral lobe of the cerebellum. On section of the alcohol-hardened brain, similar abscesses

were found in the depth of the white substance. The meninges were injected and the cerebral vessels dilated. The spinal cord showed no macroscopic alterations. Cover-slip preparations from the meninges gave a few bacilli, whereas from the brain abscesses the greatest number of characteristic and beaded forms were obtained. The heart muscle contained several abscesses. The largest occupied the wall of the left ventricle, several smaller ones, the size of miliary tubercles, occurring in other situations. Cover-slip preparations from these showed the characteristic bacilli.

The Uterus.—Several foetuses, averaging 4 millimetres in size, were contained in the two cornua of the uterus. Of these, two only were examined. They were found to be haemorrhagic, and an extravasation of blood had taken place in the membranes surrounding them. The placentæ were not haemorrhagic. The cavity of the uterus throughout, which was not occupied by the foetal structures, was covered with a gray or yellowish exudate, and several of the uterine segments which contained young were distended with gas. The condition observed here reminded one strongly of the appearances first noted in connection with the original case. Cover-slip preparations prepared from various parts of the uterine cavity showed the characteristic bacilli. They were, however, most numerous in the grayish exudate which covered the mucosa.

On May 1st, at 3.30 P. M., a grown rabbit received 0.5 cubic centimetre of the pleural fluid intravenously. Death occurred May 4th at 12 M. Convulsions were not noted in this animal.

At the autopsy the brain presented much the same appearance as in the previous one. There were, however, in addition, distinct abscesses in the olfactory lobes. The heart presented an extraordinary appearance. Through the unopened pericardial sac large numbers of abscesses were observed in both ventricular walls. These were situated below the epicardium, which was hyperæmic and covered with a sticky fluid. In the substance of the heart muscle and in the papillary muscles beneath the endocardium the abscesses averaged in size a mustard seed. They were yellow in colour and of firm consistence. One hundred and sixty were counted beneath the epicardium and the endocardium without taking into consideration those in the muscular substance itself. The liver in this case contained psorosperm nodules. In the substance of the liver there occurred a number of abscesses which in the posterior or caudal lobe reached a large size and were surrounded by a zone of necrotic liver substance. A localized peritonitis was present in the liver over the affected areas. The kidneys, spleen, uterus (non-pregnant), voluntary muscles, and joints were free from localizations of the organism. The bacterioscopical examination showed large numbers of bacilli in the substance of the abscesses.

With the exception of the instances of general infection, to be mentioned later, in no case of intravenous inoculation were abscesses absent from the brain and heart muscle. The number of abscesses

present in these situations varied in the different cases, although, as a rule, they were present in large rather than in small numbers. In one case a large spreading abscess occupied the psoas and iliacus muscles on the left side, involving almost the whole of these structures and extending into the adjacent soft tissues.

In no instance did abscesses appear in the kidneys, the spleen, the lungs, or the joints, nor in the genital organs, excepting in cases of pregnancy, as has already been mentioned. As compared, therefore, with experimental *staphylococcus-aureus* infections, these differences in localization are to be remarked. It will be recalled that in the latter abscesses are, if present anywhere else, rarely absent from the kidneys; they are not infrequently present in the heart and the voluntary muscles, less commonly in the central nervous system and other organs. It does not seem possible, therefore, to explain these differences by the facts regarding the circulation in the different organs. We incline to the view, of which we shall have more to say later, that the different organs present varying opportunities for the development of bacteria even during life, owing to the peculiar metabolism with which they are concerned, and which must modify their actual chemical composition.

In the cases previously mentioned, cover slips were made from organs which gave no evidence of the localization within them of the bacilli, and these were found uniformly not to contain the organisms. In no instance was a single bacillus met with in these preparations. In consequence of our inability to cultivate the bacillus, the study of these cases by cultures, which would have been more satisfactory and conclusive, could not be carried out.

That this organism is capable of producing actual septicæmia is proved by the following case; but, while it does not stand alone as the one instance in which a general infection was produced experimentally, it is to be regarded as exceptional in this respect:

On June 8th, at 12 M., a half-grown rabbit received 0.5 cubic centimetre of the pleural fluid of another rabbit autopsied the same day into the marginal ear vein, and succumbed during the night.

At the autopsy, which was made on June 9th, at 9 A. M., there was no excess of fluid nor any inflammatory exudate present in the peritoneal cavity. The spleen was enlarged, softened, and congested. The liver was congested and contained many superficial and deep foci of necrosis. The kidneys were swollen and the lungs congested. The pleural cavities contained a small amount of bloody fluid, the pericardium a similar fluid, while the brain was apparently normal.

Cover-slip preparations from the spleen, liver, kidneys, lungs, pleural and pericardial fluids, the heart's blood, bile, and brain—all showed large numbers of typical bacilli.

Inoculations which failed.—From time to time while the experiments described above were being conducted inoculations of the same material were made into other animals with negative results. It will be recalled that in the guinea-pig the intrapleural inoculation was successful. On the other hand, the subcutaneous injection of considerable quantities of the infectious material into these animals produced not the slightest reaction, nor did they give rise, apparently, to any inconvenience to these animals. The inoculation of relatively large amounts subcutaneously or intrapleurally into mice, whether wild or tame, were never followed by any apparent effect. Pigeons likewise showed themselves practically indifferent to the subcutaneous, intramuscular, and intraperitoneal injections of quite large quantities of the infectious material. One of the subcutaneous veins of the leg was exposed in a dog into which 1 cubic centimetre of peritoneal fluid containing many bacilli was injected. There was no immediate effect following the injection, nor did any perceptible untoward symptom develop during several weeks of observation of this animal. When we consider the great susceptibility of the rabbit, and the fact that there appeared only slight and insignificant differences in the mode of its reaction to inoculations conducted in various ways, the variations in the case of the guinea-pig appear the more remarkable, and the entire absence of susceptibility in the remaining animals which were tested worthy of remark.

THE ORGANISM AS OBTAINED FROM THE EXPERIMENTAL CASES.

In the course of the transference of the infected material from animal to animal certain variations in the appearance of the organ-

isms as they were derived from different cases were noted. These variations were never so great as to warrant the supposition that the original species was lost, or even had become, in the course of its transference, admixed with another species. The variations affected the relative numbers of short forms and long forms which might occur in any given situation, and, furthermore, the degrees to which the irregular staining, already described as characteristic of this organism, became less regular or tended entirely to disappear. For example, in the course of the successive inoculations into the pleura it was noted that the short forms of the bacilli diminished greatly in numbers, and whereas in the beginning very few solidly staining forms occurred, as time went on a larger number of non-beaded ones appeared. The total number of the latter was, however, never so great as was observed under some other conditions, as, for example, in certain cases of intravenous inoculation and in some situations in the infected uterus in the case of the intra-uterine inoculation. Closely allied with the tendency to appear as solid forms was the tendency toward increased thickness in the latter. Now and then the solid bacilli reached a thickness of at least twice that of the characteristic forms. It would further appear, from an observation made in the case of the intra-uterine inoculation, that the solid forms really represented stages toward degeneration. In this instance positive evidences of involution were obtained in those bacilli which had lost their characteristic markings. These were swollen to twice the average size and contained irregular vacuoles. In extreme cases the chromatin material was irregularly segmented and stained with varying degrees of intensity, and an appearance of knoblike projections was noted in certain of the bacilli. The latter were present in the largest number in the cases of abscesses developed after intravenous inoculation. They gave a peculiar appearance to the modified organisms, inasmuch as they were composed of very deeply staining protoplasmic masses situated at irregular intervals along the course of the rods. The swellings averaged about twice the thickness of the rods, and might be of even still greater size. They were not to be confounded with appearances

brought about by angular bends and by an irregular twisting to which the longer bacilli were more or less subject, and which might occur several times in the course of the longer forms. From the study of certain of the larger abscess formations—as, for example, in the subcutaneous tissues and in the liver—the impression was obtained that the size of the bacilli varied to a certain extent with the number present and the rapidity of their development. In the contents of the abscesses in both of these places the numbers were very great indeed, and coincidentally the shorter forms, which had more or less disappeared, now reappeared, and rather predominated over the larger ones. The changes in staining properties and those attributable to involution were not noted in the smaller forms.

PHAGOCYTOSIS.

Although in all the local exudates the bacilli were associated with pus corpuscles, the phenomenon of phagocytosis was observed in a few instances only. In the case of meningeal inoculation in which the animal lived twenty-two hours and a half a great emigration of leucocytes had occurred, and very occasionally the bacilli which for the most part stained faintly were detected in polymorpho-nuclear leucocytes. Such phenomena were very unusual in the pleura, although in a very few instances cell inclusions had taken place. In one case of intraperitoneal inoculation, in which death occurred after forty-eight hours, bacilli were observed occasionally, both within the cell protoplasm and within the nuclei of the emigrated white corpuscles. The best example of phagocytosis was observed in a case of intra-pleural inoculation of the guinea-pig, in which death occurred in about forty hours. The inflammatory exudate contained a large number of leucocytes with polymorphous nuclei, and these not infrequently included bacilli. The included forms were for the most part long, and they were curved round the interior of the cell, owing to the fact that they were often several times as long as the cell itself. The included organisms stained, as a rule, more faintly than those without cells, although there were not a few exceptions to this rule. The phagocytic pus cells appeared quite normal, and contained

bright, well-staining nuclei. It has already been mentioned that in one instance an imperfectly inclosed bacillus was observed, in which the intracellular stained more faintly than the extracellular portion.

CULTIVATION OF THE ORGANISM.

Notwithstanding the failures to cultivate this bacillus which have been mentioned in detail in the early part of the paper, further attempts were made upon the ordinary media from time to time in the course of the transference of the organisms from animal to animal, and always with the same negative results. In no instance was the slightest evidence of increase of the bacilli obtained. At this juncture it was decided to attempt their growth upon the sterile organs of the rabbit itself. With this end in view, experiments were first conducted for the purpose of determining whether or not it would be possible to isolate the various organs of the rabbit and preserve them free from contamination with extraneous micro-organisms. Very little difficulty was experienced in this direction. The hair of a rabbit, which had been killed by a sharp blow on the back of the neck, was laid by wetting it thoroughly with ninety-five-per-cent alcohol. The skin was now cut through with sterile and heated scissors, and dissected back with sterile instruments, leaving the abdominal walls, minus the skin and subcutaneous tissues, widely exposed. The latter were flushed with alcohol, a sufficient excess being used to moisten thoroughly the surface and to form small accumulation in the angles formed at the point of junction of the abdominal walls and the dissected skin. By means of instruments rendered sterile with heat the abdominal parietes were now laid back, care being exercised to avoid even contact with underlying intestines. Recently sterilized test tubes of larger calibre than those usually employed, and Petri's dishes, also recently sterilized, being close at hand, pieces of the liver, spleen, kidney, uterus, and lung were quickly excised with sterile instruments and dropped into these vessels. The heart was removed with its pericardial investment entire and placed in a Petri plate. Care was taken to avoid contact with the upper portions of the test tubes to which the cotton plug was ap-

plied. The charged plates and tubes were now placed aside for several days, and those which remained uncontaminated were inoculated with material derived from the experimental animals. It is interesting to note that only occasionally did one of these tubes show any contamination. Inoculations were made upon the sterile organs on April 2d, and the cultures, placed in the thermostat at the temperature of 37° C., were removed on the 5th of April. One of the lung tubes was found to have become contaminated by a growth of mould, and was discarded. The tubes containing the liver and the spleen had assumed a slightly opaque appearance. They were, however, entirely free from odour of putrefaction, and cover slips which were prepared showed an entire absence of micro-organisms, including even the transplanted bacilli. One of the kidney tubes was likewise free from organisms of any kind, although it had been inoculated with the *bacillus pyogenes filiformis* with the rest. A Petri plate containing a whole kidney, which had been incised in the middle and allowed to fall apart, the freshly cut surface of which had been inoculated, showed a faintly opaque, slightly greenish, elevated deposit, which, upon examination, proved to be a growth of micro-organisms similar to those which had been transplanted.

The inoculation of the heart was made between the layers of the pericardium. On removal from the thermostat there was an entire absence of odour, the organ looked only a little darker than at the time of its introduction, a similar opacity to that present on the kidney had spread over the limits of the pericardial sac, and cover-slip preparations showed a growth of organisms resembling those observed in connection with the kidney.

The inoculations of the uterus were made directly into its cavity. Removed from the thermostat, it presented a slightly greenish appearance, possessed a very faint odour—not, however, one suggesting putrefaction—and an opaque material covered the exposed ends of the inoculated tissue. Cover-slip preparations of the latter showed the same organisms as upon the two previously mentioned organs.

The previous descriptions refer to cultures under ordinary conditions. Similar ones were made in an atmosphere of hydrogen. The

anaerobic tube containing the liver showed a considerable separation of bloody fluid, but there was absolutely no development of the introduced bacilli. The surface of the lung in anaerobic culture was moist and grayish in appearance and contained numerous bacilli indistinguishable from those observed in the aerobic cultures. It can, however, be said that the growth was less abundant in the anaerobic tubes than in those exposed freely to the air. Thus it will be observed that upon the sterile organs of the rabbit the first success of our many attempts to cultivate this organism was met with.

Transplantations were now made from organ to organ, and subsequent generations were obtained—not, however, with equal success in all cases. For example, in the case of the lung a new growth appeared, but the organisms could not be grown upon this medium beyond the third generation. The uterus also permitted the development of three generations only, whereas in the case of the kidney a second generation, but not a third, was obtained. The first growth upon the organs was the most vigorous, the subsequent generations being very slight indeed, and the last hardly to be recognised by the unaided eye, but requiring the use of cover-slip preparations to demonstrate the presence and increase of the transplanted micro-organisms. On April 20th the experiments were repeated, but this time a pregnant female rabbit was employed for obtaining the material for cultures, and to those organs previously used portions of the sterile placenta were now added. The result of the inoculation of a full series of tubes derived from this animal is as follows: The lung showed a good growth, the uterus quite abundant growth, the kidney less growth than either of the two mentioned, the placenta a doubtful growth, the liver and spleen no growth. An attempt was made to cultivate the bacilli in bouillon to which a bit of sterile lung had been added, but without success. Transplantations conducted as in the previous instance gave a progressively decreasing development upon the various organs during from two to three generations, after which no further growth could be obtained. It was impossible to obtain even a second generation upon the placenta.

Although we had now succeeded in cultivating the bacillus through two or three generations, the growth obtained upon the various organs mentioned was never abundant, and the limited development of the last transplantation was not regarded as offering favourable opportunities for the further experimental study of the cultivated organisms. It is therefore of interest to note that upon the foetus of the rabbit transplantations from one of these objects to another was possible through a much larger series than in the previous cases. The method of obtaining the foetuses was similar to that employed for obtaining the sterile organs. Pregnant female rabbits were employed, and care was exercised to obtain for this purpose such as had been impregnated probably ten days to two weeks before. The foetuses which were most favourable for our purposes averaged from 5 millimetres to 1 centimetre in length. The exposed uterus was incised with sterilized instruments, the membranes separated, and then with a new set of sterile instruments the foetuses were excised and introduced into recently sterilized test tubes. After a little practice this operation could be executed so expeditiously that only in rare instances was a tube found to have been contaminated. The sterile objects were then inoculated in the usual way with material derived from the experimental cases, and now for the first time an abundant growth was obtained. After twenty-four hours in the thermostat the tissues had become darker in colour, a slight scum seemed to overspread the surface, and some discoloration could be observed in the amniotic fluid in which the foetus lay. Cover slips showed many bacilli of the length of the original forms, but differing somewhat in their staining properties. The organism was cultivated through a series of six of these objects, which represented the number of foetuses obtained from one animal. There was no perceptible difference in the growth upon the sixth as compared with that upon the first. From the sixth generation intrapleural inoculations were made with the result of causing the death of the animal in three days, with lesions which were in all respects typical. This series of experiments was repeated at a later date with essentially the same results. It should be mentioned that the organisms which had ceased to

develop in the second or third transplantation upon the lung and the uterus, when transferred from these to the foetus grew quite abundantly.

It will be recalled that among the early attempts which were made to cultivate the organism use was made of the heated and the unheated blood serum of the rabbit and of the coagulum from which the serum had separated, but with entirely negative results. After the successful experiments just mentioned it was decided to see whether or not a culture medium prepared from the organs of the rabbit upon which the bacilli grew could be used for their cultivation. With this end in view, a large pregnant female rabbit was killed by a sharp blow on the back of the neck. The abdominal and thoracic cavities were opened in the manner employed for removing the sterile organs. The heart and lungs, the kidneys, and the much-enlarged uterus, which contained eight well-developed foetuses and placentæ, were removed and carefully hashed. An equal volume of sterile water was added and they were permitted to macerate in the ice chamber for twenty-four hours. The expressed fluid was passed through a Chamberland filter. The resulting straw-coloured, clear, and sterile fluid was introduced into test tubes to one part of which liquefied two-per-cent agar-agar at 40° C. was added. These tubes were permitted to harden in a slanting position. The remainder of the fluid was kept as such. Both the solid and the fluid media were inoculated from the local exudates of experimental animals and kept under aerobic and anaerobic conditions, but in no instance was the slightest evidence obtained of development upon either.

This experiment was repeated subsequently with the difference that the infusion was made entirely from pig's foetuses, which measured each about 2 centimetres in length, but again with negative results. It may be mentioned, in passing, that as culture media for growing ordinary and, indeed, some unusual micro-organisms, both of these preparations answered exceedingly well. For example, the ordinary bacteria grew abundantly upon them, and the particular medium prepared from the pig's foetuses proved to be suitable for the

growth of the gonococcus. We shall take occasion later to draw attention to the apparent significance of this observation as bearing upon the influence of certain constituents of tissues in determining the ability to develop, and the extent of development of certain micro-organisms.

The Organism as obtained from Cultures.—The variations in size and staining which have been described in connection with the bacilli obtained from various sources in the body of infected animals were again met with in the cultures. In the earliest cultures, as, for example, upon the kidney, lung, and uterus, there was a preponderance of the smaller forms. Among these some were as small as the least of those present in the experimental animals, although the greater number corresponded to two, three, or four of the dotted segments of the original bacilli. The long, threadlike forms did not, as a rule, reach the extreme length of the originals, and a tendency to the appearance of cross divisions was noted which separated some of these into pseudo-chains. The thickness also varied, the average breadth in the cultivated forms being somewhat less than in those obtained directly from the animal. The ends of the bacilli were rounded, and the great majority of both long and short forms stained solidly. Now and again slightly thicker ones were met with, which reminded one of the thick, solidly-staining rods sometimes seen in exudates, and these not infrequently were knotted and apparently undergoing involution. In the first inoculations from the animal the transplanted bacilli could usually be distinguished from the newly developed ones. The former retained their original forms, but they stained very slightly indeed, and were apparently undergoing degeneration. More than once was there observed an outgrowth from the original bacilli, which in the older parts were undergoing degeneration, while the growing end stained brightly and sharply. The degenerated end of such specimens could at times be seen to have the regular and characteristic dotted appearance, while the growing end stained equally and solidly throughout. The bacilli tended to grow in clumps, the longer forms overlying each other at various angles, the shorter ones appearing with-

out any striking regularity, at other times large and small forms were often intermingled in the growing masses (Plate IX, Fig. 2).

It was remarkable to what extent the nuclei of the tissue cells stained sharply and well, although the tissues had been removed from the body for many days, and now were overgrown by the organisms. A condition which might be termed pseudo-phagocytosis was occasionally observed in connection with these cells. The protoplasm, and more rarely the nuclei, were invaded by the bacilli, which sometimes were closely crowded within the protoplasmic substance of the cells, and again seemed to lie in vacuoles in the nuclei. The latter structures in the affected cells stained quite brightly, while the staining of the protoplasm was not to be distinguished from that of the uninvaded cells. It is safe to say that such an appearance observed in cells derived directly from animals would be taken to indicate phagocytic inclusions. It should be mentioned, however, that the long forms were not found inclosed in the cells.

Variation in Morphology produced by Inoculation.—It is apparent from the foregoing description that there is a distinct change in the general morphology of this organism in cultures compared with the appearances observed in exudates. While in the latter solidly staining forms are unusual, in the former they are not alone the most common, but after the second or third generation the only forms to be found. This peculiar, although not altogether new, observation of modification in form and staining properties in the cultivated organism compared with the more parasitic forms might be taken to indicate contamination at the time of the first transplantation, and, finally, a complete replacement of the original by the extraneous forms. What renders this improbable, and indeed quite inconceivable, is, in the first place, the fact that the cultures obtained from various sources and at different times all behaved in the same manner; and, secondly, that the inoculation into the pleural cavity of a rabbit of the organisms derived from cultures which had the morphology described in connection with these, set up a pathological process which was indistinguishable from the origi-

nal cases, and, what is of greater importance, the micro-organisms contained in the local inflammatory process again showed the typical irregular staining of the original bacilli.

Vitality upon Cultures.—An experiment was made to determine the duration of survival of the organisms upon sterile organs. On April 7th transplantations were made to the uterus and the lung. After twenty-four hours in the thermostat, at which time a fair growth had taken place, the tubes were removed, covered with rubber caps, and placed in a cupboard in diffuse daylight. They were removed on April 26th, and were found to have dried out to some extent. Cover slips showed a small number of organisms in these cultures. Transplantations were made upon the uterus, lung, and foetus without obtaining any growth. It would therefore appear that the bacilli were incapable of surviving upon these media as long as eighteen days. From our previous experiments it is probable that the vitality is considerably less than this experiment would indicate.

THE HISTOLOGY OF THE AFFECTED ORGANS.

The Lungs.—It has been pointed out that the pathological changes occurring in the pleura and the lungs of the spontaneous case do not differ essentially from those present in the experimental cases of pleural inoculation. It will be recalled that the pleura in these instances was covered with a shaggy false membrane, and that the affected cavities contained an increased amount of red fluid. The lungs presented, in some parts, appearances indicating that they were consolidated, while elsewhere they appeared to be collapsed, and in still other places to be insufflated. The bronchi contained, at times, pluglike masses which occupied their lumina, and which often extended into and quite filled the larger branches.

We shall take up separately the various structures affected. The exudate upon the pleura consists of large numbers of cells, the principal ones being polymorpho-nuclear leucocytes, although among these there are not a few which present larger vesicular nuclei. The amount of fibrin present within this exudate is surprisingly small,

although it varied somewhat, and was least abundant in the spontaneous case. The shaggy character of the membrane is found to be due to the large numbers of closely interwoven bacilli themselves, which imprison the inflammatory cells in their meshes. It is indeed difficult to give in a simple description an adequate idea of the enormous numbers of the latter which are contained in this false membrane. The pseudo-membrane is placed directly upon the serous covering of the pleura, and not infrequently the covering endothelial cells of the latter can be made out beneath the exudate. Indeed, they give the impression of having undergone proliferation, which fact may account for the relatively large numbers of cells with vesicular nuclei contained within the exudate.

The inflammatory process extended directly through the pleura into the substance of the lung. The superficial layer of lung alveoli, to a depth of two or three rows, is quite if not completely filled with a material resembling that present upon the pleura. Here, again, one is struck with the large numbers of bacteria present; and the manner of their growth through the pleural membrane can be readily determined. There is a direct extension from the surface into the most superficially placed of the alveoli, the bacilli occupying parallel rows and growing at right angles to the surface of the lung. They accommodate themselves accurately to the size of the invaded alveoli, often filling entirely by their growth the superficial layer, leaving practically no room for the exudative cells, which in these situations occupy the alveolar walls. Although the shapes of the bacterial masses here present appear to have been determined by the forms of the alveoli, it is interesting to note that the growth upon the surface of the pleura, which is in direct connection with the organisms in the alveoli beneath, presents much the same grouping, and strikingly suggests sheaves of wheat. Deeper in the substance of the lung the alveoli contain fewer bacilli, although a not inconsiderable number, and more of the inflammatory products. Where the bacilli and exudative cells are less abundant, fibrin appears more frequently in the inflamed tissue.

The affection of the bronchi is found to be far greater than was

evident at the autopsies. In no case was such affection absent, and frequently the bronchitis is a striking feature of the process in the lungs. Both large and small bronchi are affected, sometimes alone, but oftener with the involvement of the adjacent lung substance. The affected bronchi are, as a rule, completely plugged by a mixture of cells and bacteria. The predominating cells are again the polymorpho-nuclear leucocytes; and the bacteria varied in numbers in different situations within the bronchi. They are accumulated in greatest numbers directly in contact with the mucous membrane, at which situation they are disposed with great regularity. In the more central portions of the exudate they are fewer in number and more irregularly distributed. The grouping in connection with the mucous membrane is the same as that observed upon the surface of the pleura. The bacilli are disposed in parallel lines, which appear to start from an expanded base at the mucous membrane and to meet in a tolerably sharp point a short distance within the lumen of the tube, thus producing a series of conical elevations. The epithelium of the mucous membrane may be preserved, although it is always infiltrated with leucocytes. Now and then it has disappeared, and small defects exist in the mucous membrane; more rarely separated epithelial cells are found in the interior of the bronchi among the inflammatory elements and bacilli. The infiltration of the bronchi extends into the submucous, the muscular, and the adventitial coats, and thence, in certain instances, into the surrounding lung substance. The infiltration is accompanied with no small amount of cell destruction, as is shown by the nuclear detritus present in these situations. The size of the consolidated areas in the lung varies very much, and only rarely reaches the extent of the larger part of a lobe. In the case of a "lobar" consolidation there is a great want of uniformity in the appearance presented by the affected tissue. Some parts of it are firm and others are more loose in texture, presenting the picture of a number of smaller lobular foci of solidification, which have been more or less closely united by an intervening, loose inflammatory exudate. This appearance accords well with the involvement of the bronchi, which is greatest in the

areas of firm hepatization, and from which the lung parenchyma seems to have been invaded. The amount of cell destruction in the firmly-consolidated portions is often very great, and under the microscope may present an appearance much resembling the caseation of a tuberculous pneumonia. The bacteria are present along with the infiltration, and, indeed, appearances frequently suggest a reverse order of growth to that described; that is, from the affected lung tissue in which a relatively few bacilli are present the bronchi have been invaded secondarily, the rich development of the organisms occurring where they have free access to air—namely, next to the mucous membrane. The invasion of the bronchi in certain of the experimental cases could be observed to extend and ramify with the subdivisions of the larger bronchus. This has reference to the smaller tubes, which soon became lost in the lung parenchyma. Pictures showing the bronchioles, atria, and air cells which contained inflammatory products were seen. In the terminal bronchus the lining epithelium, now somewhat flattened, was not infrequently still present, whereas the exudate in the other structures invaded both the lumina and the alveolar walls, and thus obliterated the lining epithelium. In one instance we observed a continuous growth of the organisms, which extended through the ultimate structures just mentioned and gave rise, by the twisted character of its rounded contours, to an appearance suggesting the ramifications of a thick, corded rope. About some of the areas of broncho-pneumonia small foci of lung collapse occur. All of the structures about the bronchi, as well as the parenchyma of the lung itself, may be invaded with bacteria. It has already been mentioned that the several coats of the smaller bronchi may be the seat of the bacterial development, and in the larger bronchi neither the perichondrium nor the cartilage is spared, although the former is much more affected than the latter. The bacilli rather than the inflammatory cells invade the cartilage, and they are found both in the superficial and in the deeper layers between the cells as single bacilli or as small groups, occupying, apparently, the lymph spaces, and never, so far as was observed, actually invading the cells themselves.

The blood vessels in the areas of affected lung show interesting changes. A continuous extension of the inflammatory process from the lung substance, through the various coats of the vessels, including the internal coat, may take place, in which case large numbers of fragmented nuclei are present, both in the intima and in the lumina of the vessels. Such affected vessels contain thrombi of fibrin and leucocytes, which either partially or completely occlude their lumina. This inflammation of the vessels occurs in very large branches of both the pulmonary artery and the pulmonary vein. Another condition of really greater interest occurs very extensively in the spontaneous case, and not infrequently in the experimental cases. The vessels in the regions of the inflamed lung tissues show an increase of cells in the internal coat, which in the larger vessels distinctly narrow their lumina, while by the same process the smaller branches may be completely obliterated. The proliferated cells are for the most part large, possess vesicular nuclei, and resemble in size, staining properties, and relation of nucleus to protoplasm, the large mononuclear elements found normally in the blood. Among these a few definite polymorpho-nuclear leucocytes and an occasional small cell with deeply staining, round nucleus exist. An endothelial covering could often be made out, at least in the medium and larger-sized vessels, and the proliferated cells be followed to the internal elastic lamella, where they usually stopped. In not every case could a layer of flattened endothelial cells be found to cover the new cells, and the latter then might project directly into the lumina of the vessels. In very small vessels the flattening of the most superficial layer of cells is, as a rule, not present at all. Among the polymorpho-nuclear leucocytes within the blood of these vessels a large proportion of large mononuclear cells occur. At times these cells, apparently free in the current, communicate with the new cells in the intima, and, although we do not consider the evidence as sufficient, yet the possibility of derivation from this source of the increased mononuclear elements is too great to be overlooked.

The multiplication of the cells in the intima is by indirect cell division. The number of karyokinetic figures met with is surpris-

ingly large, and the figures are rarely seen in the depth of the new cells next the internal elastic lamella. From this it follows that the division takes place in the superficial rather than in the deeper cells. Indeed, the dividing cells are often the ones immediately next the lumen of the vessel, and in character resemble the vascular endothelium in the appearance of their nuclei only. We can not see why they may not have been derived from the endothelial cells into which they could readily be converted, in appearance at least, by simply becoming flattened. It is of still greater interest to find among the free mononuclear and polymorpho-nuclear elements in the blood current similar cells to those in the intima which are undergoing mitosis. The proliferated cells now and then arranged themselves in parallel lines at right angles to the lumina of the vessels, these lines being separated from one another by pale, apparently structureless fibrils without nuclei. Sections of the vessel which show the entire circumference indicate that the growth affects the vessel wall in its whole extent. That this process is obliterative in its tendencies, the study of vessels of various size leaves no doubt. It can only be surmised that in the end actual fibrillated connective tissue might result, and the final appearance be that of the commonly observed endarteritis and endophlebitis obliterans. The vessels which show changes in the internal coat are in the neighbourhood of the affected lung tissue; they are not, however, the seat of active changes in their other coats. So far as can be determined, they are not the seat of actual bacterial invasion.

The lymphatics of the lung, particularly those which run in the interlobular tissue, are greatly dilated, contain increased numbers of leucocytes, and not uncommonly fibrinous thrombi.

The bacilli are present in considerable numbers in all of the inflamed tissues, not excepting the walls and the lumina of the blood vessels, in which acute inflammatory changes (peri-arteritis, mesarteritis, endarteritis, etc.) associated with cell destruction were observed.

The staining agents which were employed in the study of the lung, as well as the tissues subsequently to be described, were haematoxylin and eosin and carmine for the tissue elements, fuchsin

and gentian violet for the bacteria, and Weigert's fibrin stain for the demonstration of fibrin. The most satisfactory results for demonstrating the bacteria were obtained with the methods which I had previously recommended for the staining of the typhoid bacillus in tissues.* It is as follows: Stain for a few minutes in Stirling's gentian violet (gentian violet, 5 grammes; alcohol, 10 cubic centimetres; aniline oil, 2 cubic centimetres; water, 88 cubic centimetres; filter), or one-per-cent carbolic fuchsin; place in 1 to 1,000 acetic-acid solution for some minutes, dehydrate and partially decolourize in ninety-five-per-cent alcohol, transfer to the slide, blot, and then add oil of cloves to clear and differentiate. Change the oil several times until the desired differentiation is obtained.

By the use of either of these stains, but particularly of the violet, the bacilli stain sharply and in many instances show the irregular appearances which constitute one of their characteristic features.

The Heart.—In this connection it will be necessary to treat of the lesions as they affect, first, the pericardium, and, second, the muscular substance.

Although some doubt was expressed as to whether in the case of the lung the bronchial infection preceded or followed the disease of the lung parenchyma, in the heart the two places of localization of the infectious material and the resulting inflammatory changes can be readily explained by the mode of distribution of the micro-organisms. For example, the pericardial infection occurred through direct extension from the inflamed pleura, whereas the foci situated in the depth of the muscle resulted from the vascular distribution of the bacilli. There is one exception to this statement—namely, in the case mentioned in which following the pleural inoculation there was in association with the pericarditis a single nodule or abscess present in the ventricular wall. That this was produced by direct extension from the pericardium can not be excluded, although it is equally impossible to say that the bacilli were not carried to the muscle either by the lymph or the blood channels.

* *The Johns Hopkins Hospital Reports*, vol. v, p. 366.

The pathological process present in the pericardium is strikingly similar to that upon the pleura. We have again a pseudo-membrane which varies in thickness, presents a shaggy appearance, and contains large numbers of bacilli. The same deception with reference to the fibrinous character of the exudate prevails here as in the case of the pleura, the large numbers of inflammatory cells being held together principally by the dense network of bacteria. A few small masses of fibrillated fibrin could, however, be seen in the exudate. The exuded cells are principally polymorpho-nuclear leucocytes, among which, however, not a few cells with single vesicular nuclei are to be found. The endothelial layer of the pericardium is generally not to be made out distinctly, because, perhaps, of the great infiltration; yet, in some places where the exudate is less thick, the endothelial cells can be seen to be preserved beneath the latter. The infiltration in those situations in which areolar and adipose tissue occurred penetrates a variable depth into these, and in those places in which there is no adipose layer between the serous membrane and the muscle an increase of nuclei has occurred between the muscle fibres. Some of the increased cells are certainly leucocytes; others appear to be proliferated, fixed tissue cells. Indeed, the latter seem to be derived, in part at least, from the pre-existing muscle nuclei. Deeper down in the substance of the heart, particularly about the larger vessels which enter its substance and around which the connective tissue is thicker than elsewhere, a moderate infiltration with small round cells and larger cells with vesicular nuclei exists. Thrombi occur in the lymphatics and veins of the pericardium, and many of the small veins are crowded with white blood-corpuses. It should be mentioned that the sheaflike appearance observed in the massed bacilli in the lung and pleura was not to be made out in the pericardium. In this situation the organisms formed a fine and small meshed felted network in which many presented the characteristic dotted appearance.

The focal localizations of the bacilli within the muscular substance occur in all parts of the ventricular walls. They may be present just beneath the endocardium or just beneath the pericar-

dium. Where they occur beneath the pericardium an exudate of opaque and sticky character occurs over them, giving rise to localized foci of acute pericarditis. The abscesses occur singly as well as in small groups. As a rule, the single ones exceed in size a millet seed and can be distinguished from abscesses caused by the pus organisms by their firmness and the absence of tendency to liquefaction on the part of the tissues. Two appearances, apparently quite dissimilar, are obtained, according to whether the affected heart muscle is stained with ordinary nuclear dyes, such as hæmatoxylin, or with the anilines, which stain the bacilli rather than the tissues. For the study of the tissue elements the best results were obtained from the use of hæmatoxylin and eosin. The specimens stained in this way show in the affected areas large numbers of emigrated cells disposed between the muscle fibres, often in parallel or slightly angular lines. The outlines of the abscess are round, oval, or slightly more elongated. In the affected areas the actual number of muscle cells is diminished, at least so far as the demonstration of their presence is concerned. From the thinned and compressed appearance of the muscle cells it would appear as if there had been an actual loss in numbers; still, where the infiltration is so great it may be that there is an obscuring of the fibres and not an actual disappearance. The atrophied and compressed fibres described are modified in their staining properties. They present a homogeneous and hyaline appearance, have lost their striation, and are devoid of nuclei. They are evidently necrotic.

This change in the muscle fibres is not limited to the immediate contents of the abscesses, but extends a variable distance from the periphery, and may affect groups of fibres quite removed. The affected fibres not directly in the abscess contents may still possess colourable nuclei, although these are more or less modified. They are smaller than the normal nuclei and take a very deep and opaque hæmatoxylin stain. It is more exceptional to find in the muscle fibres within the abscess the retention of their nuclei. Fragmented nuclei are present in relatively small numbers, and there would appear to be but little change in the fixed cells about the affected

areas. The gentian-violet stain shows a most extraordinary development of the bacilli in the abscesses. They surround the individual muscle fibres, which are pale and colourless, and are identified by their form and, occasionally, where these are retained, by their nuclei.

The bacilli are, however, not limited to the capillaries, but, corresponding to the points of atrophied or even deficient muscle fibres, they form an interlacing, close-meshed network within the tissue. Their thickest development is not in the centre of the abscess but is at the periphery, the network in the centre being much more delicate than at the outer zone. The bacilli do not end abruptly at this outer zone, but may be followed in the form of small groups and even singly between the adjacent muscle fibres, and apparently here they are chiefly within vessels. It can not be stated that there is a growth directly into the muscle fibres, although appearances suggestive of this were observed. The aniline stain also brings out the fact, less noticeable in the tissue stained for the tissue elements alone, that there is a much more extensive destruction of cells, principally through a fragmentation of their nuclei, than was before evident. These fragmented and necrotic cells lie in the periphery of the abscess and occupy definite spaces between the muscle fibres, which may be capillaries or lymphatics. Intermingled with the cellular detritus there are many of the characteristic bacilli.

From the descriptions of the lesions in the pericardium as well as in the heart's substance, it will be noted that in the first place the changes are of the nature of an acute serositis associated with a moderate interstitial myocarditis, and that, as compared with staphylococcus abscesses, the abscess formation here is peculiar, owing to the absence of evidences of softening of the tissues and because of the localization of the bacilli in the periphery of the cellular infiltration rather than in its centre. The degenerations produced in the muscle cells themselves are very extensive, and vascular changes of a proliferative character, similar to, although less extensive than, those in the lungs, take place. Thrombi occur both in the vessels beneath the pericardium and within the muscular substance, but

undoubted examples of proliferation of the cells of the intima have not been found associated with pericardial affection alone.

The Brain.—The microscopical study of the cases of meningeal infection show that the lesions are not limited to the membranes, but that the brain substance suffers as well. Even in the earliest cases—for example, in the one in which death occurred twelve hours after inoculation—the lesions, both in the meninges and in the cerebral cortex, are well advanced.

Corresponding to the seat of inoculation there is a rich infiltration of the leptomeninges, which is in the form partly of tolerably circumscribed nodules of pus cells and partly of a diffuse infiltration with the same. The thickness of the exudate is less in the diffusely infiltrated parts. The adjacent brain substance is not the seat of abscess formation. On the other hand, for the distance of 1 millimetre, or thereabouts, the cerebral cortex is more than normally condensed, stains deeply in eosin, has suffered disappearance of tissue cells, and a moderate infiltration with polymorpho-nuclear leucocytes. Bacilli are in this case fairly abundant only, and are limited, so far as the sections indicate, to the meninges. Where the duration of life was greater, as in the case of the animal which survived a subdural inoculation for twenty-two hours, the lesions are more advanced. The meninges show similar but diffuse infiltration, whereas the underlying brain substance is greatly injured.

The superficial cerebral cortex, corresponding to the seat of inoculation and extending several millimetres to each side of it, is completely necrotic, devoid of well-preserved cells, and contains fragmented nuclei. In the midst of this necrotic material a small abscess was found. The inflammation extends directly into the lateral ventricle of the inoculated side, although the brain substance in its entire depth was not so greatly modified as that near the seat of inoculation. A continuous growth of bacilli, which in the deeper parts is very slight in amount, has penetrated into the ventricle. The choroid plexus is greatly infiltrated with pus cells, and these, mixed with red blood corpuscles, also occur in the ventricle where a great increase in the number of bacilli has taken place. The ependymal

epithelium appears intact. A surprising feature of this case was noted in the cortex just beneath the seat of inoculation, where a triangular gas cavity, 2·5 millimetres in diameter in its widest part, existed. The walls of this are composed of the condensed and necrotic brain substance in which a very slight invasion with leucocytes has occurred. The number of bacilli in the pia mater and in the adjacent brain substance is very great indeed, but the greatest accumulation occurs in the condensed tissue forming the walls of the gas cavity. Although fragmented nuclei are abundant in the necrotic brain substance, it is remarkable how few emigrated cells occur in this situation.

The brain abscesses produced by the intravenous mode of inoculation varied, as was stated, in the number as well as in the size of the individual abscesses. They differ also in appearance according to the manner in which the sections containing them are stained. Hæmatoxylin and eosin show the presence of considerable numbers of polymorpho-nuclear leucocytes in the affected areas. The smaller abscesses are sharply circumscribed by the surrounding brain substance, while the larger ones spread in a peculiar and interesting way and are less sharply limited. Those abscesses the contours of which are definite, are packed so closely with leucocytes that there appears to be an entire absence of intercellular material of any kind.

On the other hand, the larger and irregularly defined ones show smaller collections of leucocytes irregularly distributed throughout the abscesses and frequently well separated from one another by intervening, faintly fibrillated material, which by this stain could be seen to be composed of masses of bacteria. The larger accumulations of leucocytes occur in the centres, the smaller eccentrically, and the periphery frequently is entirely or quite devoid of them (Plate X, Figs. 3 and 4). The deeper-placed abscesses are separated at times by a very narrow strip of tissue from the interior of the ventricles, and in those cases in which the abscesses approach the ventricle an acute ependymitis always exists. The brain tissue about the abscesses is greatly altered. Where the abscesses are spreading

in character there is extensive necrosis of cells with fragmentation of nuclei, amounting at times to complete destruction of the adjacent brain substance.

The further removed from the abscess the affected tissue lay, the less intense the effects, until only increased granulation of the protoplasm of the cells distinguish the injured from the normally staining ones. Glia cells and ganglion cells both suffered, although the protoplasmic changes mentioned were observed in connection with the ganglion cells. The blood vessels, particularly the capillaries and smaller veins and arteries, show varying degrees of involvement. Those most affected are infiltrated with cells, many of which are fragmented, the resulting detritus often following the perivascular lymph spaces and appearing also within the lumina of the vessels, which usually are thrombosed. The larger vessels, and the ones at the same time least affected, show on a small scale the increase of intimal cells similar to that described in the lung.

The relation of the bacilli to the circumscribed abscesses is that of a rather indiscriminate mingling of organisms and cells. Between the closely packed emigrated cells aniline stains show a considerable number of typical rods. On the other hand, in those abscesses which are less circumscribed the bacilli form only tolerably dense masses in the centre, the greatest growth being in the periphery, where the organisms invade the tissues in parallel lines, forming a continuous envelope which surrounds the accumulated leucocytes (Plate X, Fig. 3). Higher powers of the microscope show a finer invasion still, extending into the adjacent brain substance for a considerable distance beyond the continuous envelope mentioned. Single bacilli also push their way into the cortical substance between the nerve cells, often surround these, and apparently lie within their perivascular spaces (Plate X, Fig. 4). The tissue in which the large masses of organisms are contained shows complete absence of staining, whereas that in which the single forms occur exhibits only the protoplasmic alterations of the nerve cells already described. The micro-organisms appear capable of invading the capillary walls and of gaining entrance directly into the interior of the vessels, in which instance fragmenta-

tion of the nuclei of the endothelium results. The meninges do not, as a rule, suffer; the most discovered being a slight increase in the number of cells in the leptomeninges just over the superficially placed miliary abscesses.

This series of cases illustrates the variety of lesions which this organism is capable of producing when it finds its way into the central nervous system. We have as its earliest effect the acute inflammatory affections associated with a moderate increase of the number of organisms introduced, and other lesions which, from their nature and from the absence of the bacilli within them, would appear to be the result not of the bacterial invasion as such, but of the action of some toxic material which they produce. The changes observed in the cases of rapid death succeeding subdural inoculation are of this nature. The effects of the rapid development of the bacilli would appear to be rather of an actual necrotizing nature, such as we see in the second case of meningeal inoculation and in those cases in which there is a diffuse invasion of the brain by the organisms. In this necrotic tissue there may be an entire absence of stainable nuclei, and the intensity of the action of the poison is illustrated not only by this fact, but also by the nearly complete absence of emigrated cells. An interesting phenomenon is the ability of the organism to produce gas within the cerebral tissues, as is shown by the cavity described in connection with one of the meningeal cases. This case is further remarkable from the fact of the growth of the bacilli from the surface of the brain directly through its substance into the ventricle. The disposition of the bacilli in some of the abscesses is worthy of special note. If one of these be compared with experimental staphylococcus abscesses, the difference in the localization of the organisms becomes at once apparent. In the latter there is usually a central mass of bacteria about which the emigrated cells accumulate, and only smaller groups of organisms occur throughout the abscess contents intermingled with the pus cells. The peculiarity of the distribution which we have described is found in the extensive peripheral invasion of bacilli away from the centrally accumulated masses of pus cells next to which they are not present in

any considerable numbers. Attention has already been drawn to the striking regularity of growth exhibited by this bacillus. It is not better shown in any situation than in the central nervous system, where it forms palisadelike inclosures of the emigrated cells.

The Liver.—The histological examination of the liver in those cases in which abscesses were present in this viscus indicates that the lesions are of several distinct varieties. The structure of the abscesses themselves is modified only by the difference of the tissue in which they are located as compared with those already described in other places. There is a rich development of bacteria within the affected area, usually more marked in the periphery than in the centre. The bacteria occupy especially the capillaries, although where the liver tissue is much damaged, as is likely to be the case near the centre of the focus, they grow indiscriminately through the tissues. A rich invasion with polymorpho-nuclear leucocytes, which are distributed quite uniformly in the affected foci, has taken place. In most instances there has been great necrosis of the involved liver tissue, which is shown by the absence of definite staining and the great numbers of fragmented nuclei in these situations. Indeed, under the low power, the appearance in the haematoxylin- and eosin-stained preparations is very suggestive of caseation. Similar appearances, it may be recalled, have been described in connection with certain broncho-pneumonic patches and brain lesions.

The larger blood vessels near the abscesses are thrombosed usually with fibrinous coagula, but now and then a large branch of the portal vein, or more rarely of the hepatic vein, has been invaded by the bacteria, which may completely fill its lumen. Single abscesses exist, although there is greater tendency to the occurrence of groups, without, however, actual confluence taking place. On the other hand, the abscesses are frequently united with one another by irregularly-sized areas of necrosed liver tissue.

The necrosis of the liver cells is very striking, and consists of the conversion of larger and smaller areas of the liver substance into an irregular staining and appearing tissue. The cell protoplasm is

greatly modified; it has become homogeneous and often contains large vacuoles. The arrangement of the cells in rows is much disturbed; in many the cells have lost their connection with each other; the capillaries have collapsed and may be entirely indistinguishable. Again, in places the liver cells have disintegrated and even disappeared, leaving an irregular meshwork of hyaline appearance which may represent the modified remains of capillary walls, although nuclei are not to be made out in them. Some of the liver cells with altered protoplasm retain their nuclei, but these are shrivelled, contracted, and irregular in outline. Others contain fragments of nuclei, and into still others several polymorpho-nuclear leucocytes have wandered. Occasionally a haemorrhage has taken place into such an altered area of tissue. The whole, or nearly the whole, of a lobule may be included in the necrosis, which, from the number of leucocytes present, would appear to be strongly chemotactic. The liver cells generally are the seat of fatty changes. No bacilli were demonstrable in these necrotic foci. Similar forms of hyaline necrosis of liver cells not infrequently occur at considerable distances from the abscess formation, and indeed they have been frequently found in the liver both of rabbits and of guinea-pigs in which pleural inoculations had been made and in which there was an entire absence of localization of bacilli within the liver substance.

Still another form of necrosis was occasionally met with in portions of the liver more or less removed from the abscesses. In such places the capillaries are entirely occluded by a rich and thick growth of the bacilli, which often assume a convoluted appearance, and which, in properly stained specimens, give to these situations the appearance suggestive of a liver in which the blood vessels have been injected. The rows of liver cells between these capillaries refuse to stain at all excepting to a slight extent in eosin. The nuclei of the cells are usually so pale as hardly to be distinguished, and are frequently entirely wanting. A very few nuclear fragments exist among these cells. The appearance here suggested is of an anæmic (coagulation) necrosis. The capillary blood vessels immediately surrounding the abscesses are greatly dilated and turgidly filled with

blood, the rows of liver cells being correspondingly compressed and narrowed.

The effect of the larger abscesses upon the surrounding liver tissue is to compress greatly the latter, causing the liver cells to become elongated and spindle-shaped. There is no suggestion whatever of fibrous encapsulation. The exudate upon the capsule of the liver, which was always present where the abscesses came to the surface, consisted of polymorpho-nuclear leucocytes, bacilli in great numbers and fibrin. The inflammation from within could be observed to pass directly through the capsule of the liver and thus to infect the peritoneal surface. In some cases an accumulation of inflammatory cells took place between the liver parenchyma and the capsule, and subsequently it seemed to penetrate the latter. Where this occurs the capsule itself is densely infiltrated with cells, and its endothelial covering either entirely destroyed or so obscured as not to be made out.

It is worthy of note that the intravenous inoculation of the bacilli did not always result in the production of definite abscesses in the liver, and that in those cases in which they did appear there was evidence of previous disease of this organ. For example, either definite psorosperm nodules existed, or a well-marked cirrhosis, which might have been taken to have resulted from the presence of parasites or from the action of other injurious substances, the presence of which within the sections could not certainly be demonstrated.

The lesions in the liver may be considered as representing three distinct types: First, abscess formation which is attributable to the localization of the bacilli within the tissues, the destruction of the latter, and the emigration of large numbers of leucocytes from the blood; second, more diffuse necrotic lesions, due not to the direct action of the bacilli, but produced through the presence of the toxic products of their growth; third, a form of anæmic necrosis caused by extensive obstructions of the blood capillaries by an abundant growth of bacilli in the affected part.

The Peritonæum.—The character of the diffuse peritoneal exudate has already been described in connection with the liver, and it will

be recalled that it consists of a mixture of emigrated white blood-corpuses, bacilli and a small amount of fibrin. Where the inoculations were made directly into the peritoneal cavity and the incubation period was greater, a diffuse and moist peritonitis did not, as a rule, occur, but small nodules of grayish or yellowish colour were found upon the parietal and visceral coats. Sections carried through these showed a somewhat different structure from those described. The exudate in these cases is firmer than in the more acute ones, and under the microscope it is found to consist of cells and bacilli, with the complete or almost entire absence of fibrin. There is not so great a preponderance of leucocytes present, many of the cells having the characters of the small round elements and of epithelioid cells. The thickness of the exudate reaches 2 or 3 millimetres, and it is placed directly upon the serous coat, the endothelial layer of which is demonstrable in places beneath it.

Amid the inflammatory elements there are the greatest numbers of bacilli. These form a felted network which in haematoxylin-stained specimens might be mistaken for finely fibrillated fibrin. More circumscribed accumulations of cells occur within the general exudate, and have the size and appearance of small discrete abscesses. In the centres of some of these, large masses of bacilli, showing the peculiar sheaflike appearance already described, are to be observed. The leucocytes are massed about these, being fewer in the centre than in the periphery. The muscle underlying the serosa is infiltrated to a small extent with polymorpho-nuclear cells. On the other hand, there has been an increase in the nuclei of the sarcolemma. The blood vessels contain an increased number of leucocytes, and the arteries show upon the side nearest the inflamed peritonæum a proliferation of intimal cells similar to, although less extensive than, those described in the lung. Small thrombi are met with in the veins, the walls of which are free from thickening, such as is present in the adjacent arteries.

The Subcutaneous Tissues and Muscles.—The inflammation of the subcutaneous tissues does not in any instance reach the skin surface. The corium itself is very slightly affected as compared with the

subcutaneous connective tissue. The lesions here are in the very acute cases extreme, and consist of oedema and an extensive destruction of cell nuclei with a moderate invasion only of polymorpho-nuclear leucocytes. In the deeper layers of the subcutaneous tissue the destruction of cells is less and the emigration greater. The adipose tissue is peculiarly affected. It appears as if the fat had been liberated from the cells containing it and had aggregated in tissue spaces lined with endothelium, the intervening connective tissue of which is densely infiltrated with cells. In the more chronic cases there is less oedema and less fragmentation of cell nuclei, and indeed less cell infiltration. Just before the superficial muscle is encountered the emigrated and fragmented cells are gathered in very great numbers, presenting the appearance of a definite abscess. The walls of larger veins are completely necrotized and the veins themselves are thrombosed. The arteries may show little change, or fragmentation of a few muscle cells and many intimal cells; they may not, however, be occluded. The affection in the arteries is greatest on the side next to the inflamed part.

The muscle shows varying degrees of affection. Immediately within the infiltrated area it is hyaline and has lost its striation as well as its nuclei. The intermuscular septa are densely infiltrated with polymorpho-nuclear cells which frequently invade the muscle itself. In long sections of the muscle the affected fibres may be seen to be undergoing a gradual metamorphosis with the production of a material resembling hyaline, which exists at times in the form of drops. This muscle shows a peculiar reaction to haematoxylin, which stains in particles and lines, suggesting in a measure the striations of the original fibres. The greatest accumulation of polymorpho-nuclear cells takes place beneath the muscle in the deeper fascia, where they are present in such great numbers as to obscure the remainder of the tissue.

The relation of the bacilli to the affected subcutaneous tissue and muscle is as follows: The superficial oedematous connective tissue in which such great numbers of fragmented nuclei occur is entirely free of bacilli. The latter do, however, occur in the deeper and more in-

filtrated parts, where they dispose themselves in wavy, parallel lines, corresponding with the lines of the curly fibrous tissue with which they might readily be confounded. They evidently lie in the spaces between the fibres. The numbers here are enormous, but their greatest development is reached between the affected muscle fibres, which they entirely surround and inclose, and into which they may, in rare instances, be seen to penetrate. They are present again, intermingled in an indefinite way with the pus cells of the deeper abscess formation. Fibrin was not made out in either the subcutaneous tissues, the muscle, or the deeper cellular accumulations.

The Uterus.—In the case of spontaneous death the extent of affection in this organ varies with the local conditions. In those situations in which decidua and placenta are entirely wanting the invasion of bacilli is far less than elsewhere. The surface epithelium is in these situations usually intact, and a thin exudate, composed chiefly of cells, lies upon the surface. The glands are not particularly dilated, but the epithelium which lines them is for the most part desquamated and even degenerated. It very rarely happens that a gland has retained the epithelium in contact with its walls. Among the few preserved cells within the mass inside the gland there are many fragmented nuclei, and occasionally a polymorpho-nuclear cell. The most striking feature is the relation of the bacilli to these structures. The growth upon the surface is fairly abundant and intermingled with the cellular exudate. On the other hand, all the glands have been invaded by a rich bacillary growth, which has interposed itself between the masses of degenerated epithelium and the gland walls. An outline of each gland can be obtained from the growth within it, and here again we notice a more marked tendency still to grow in parallel lines, producing sheaflike masses. (Plate X, Fig. 5). The underlying tissues of the mucosa and of the muscle are much less affected, and show at most a slight accumulation of fragmented cells and a few bacilli in their substance.

A different picture is presented in those situations in which the decidua still exists within the mucous membrane. Sections were carried through various segments in which the thickness of mucous

menibrane indicated that the decidua was present, and it may be noted that in none of these did the decidua extend throughout the entire circumference of the mucous membrane. It usually occupied about two thirds of the circumference, leaving the remaining one third of mucous membrane provided with quite normal-appearing glands, although the superficial epithelium over the latter was rarely to be made out. A distinct separation of the decidua into a compact and spongy layer was, as a rule, impossible; occasionally a layer of closely packed cells with prominent and often multiple nuclei could be seen upon the outer surface of the membrane. For the most part, the outermost layer consists of a wide-meshed vascular tissue in which a few remnants of gland structures can be discerned. The walls of the venous sinuses and arteries are composed of quite large cells, with vesicular nuclei, which are separated by an attenuated, quite homogeneous, intracellular substance, and they are infiltrated with smaller cells, containing more deeply staining nuclei. In the deeper parts the glands are more numerous and better preserved, although they are often dilated. The tissue which separates the glands from one another presents in general the same characters as that described as existing between the blood vessels of the upper layer. The normal stroma of the mucous membrane is entirely absent in the situations of decidua formation, although it is present in the adjacent and unchanged mucosa. The blood vessels beneath the mucous membrane are enormously dilated, and the decidual vessels turgidly swollen and filled with blood. The relation of the microorganisms to these structures of the uterus is of the most intimate character. In the decidua the bacilli seem to have found most favourable opportunities for development, and they have spread out freely in the intervacular cellular tissue and penetrated throughout the depth of the transformed mucous membrane. The bacilli are not absent from the blood vessels, although they do not occur in them with great frequency; yet what appears to be a section of an arterial segment is quite filled with a bacillary mass. Wherever glands remain, there the largest single aggregations of the organisms are found. In their free spaces a return of the tendency to grow in

sheaflike masses is always apparent. Within the decidua itself the growth is more irregular, producing a felted network of organisms. In that portion of the mucosa in which actual decidua was absent the development of the bacilli is also very marked; and in these portions of the uterus the muscle shows greater changes, particularly of infiltration and cellular destruction, associated with a slight but certain invasion of the micro-organisms. Inflammatory cells, consisting chiefly of polymorpho-nuclear leucocytes, occur in considerable numbers in the affected decidua and mucosa, while they are present in increased numbers in the circulating blood within the vascular channels. Thrombi, composed of hyaline fibrin mixed occasionally with leucocytes, occupy many of the deeper vessels of the decidua, the mucous membrane, and the muscle.

The dilated segments which are described in the earlier part of this paper, and which, it is believed, owe their peculiar appearances to the presence of a gas, are now seen to be greatly attenuated, the thinnest portion being reduced almost to a line. The mucous membrane in the less affected parts show numerous glands, and in the most highly affected part the merest remnants of these structures. Bacilli are tolerably abundant throughout the mucous membrane. The points of greatest attenuation are opposite the attached end—that is, in the unattached portion of the uterus. The mucous membrane and underlying structures of the attached end are several times thicker than the greatly attenuated tissues of the unattached end. In the latter situation the muscular coat suffers an extension as great almost as the mucous membrane. Evidently the support afforded by the abdominal tissues of the attached side offered an obstacle to the dilating influence of the accumulated gas, which was exerted with greatest effect upon the free portions of the affected segments.

If we turn now to the experimental cases we shall find much the same condition as we have described in this one. The study of these cases has been limited to the uterus itself, and has not extended to the tissues of the fœtuses which remained. It is found that the decidua has essentially the same structure, occupies the same position in the mucous membrane as in the natural case, and

has afforded a similarly favourable opportunity for the development of the bacteria. In one class of cases (intravenous inoculation) these were brought to it by the circulating blood, notwithstanding which fact they are not found abundantly within the larger vessels. They are probably often in the capillaries, although they are very exceptionally found in definite veins or arteries. The outermost layer of the decidua frequently presents an extensive necrotic appearance, and the blood vessels are thrombosed in these portions, and also, although to a less extent, in the deeper layers. The superficial parts of the decidua contain but few glands, whereas the deeper parts contain many more, and these are usually dilated. There seems to be no special difference to be made out in the localizations here, as compared with those of the spontaneous case, although it may be remarked that in actual numbers the bacilli seemed to exceed those present in the first instance. The intervening portions of the uterus, between the segments which contain decidua, are much more nearly normal in appearance; and, as in the original case, these are parts of the mucous membrane which are not at all concerned in the pregnancy. Although the micro-organisms are present within the mucous membrane in these latter situations, they have not developed to the same extent as in the former, nor have they, apparently, produced the same damage. Still, it should be remarked that the gland epithelium itself has suffered desquamation and degeneration. The histological appearance presented by the dilated pouches in the experimental case in which gas was believed to be present in the uterus is similar to that described in the spontaneous case, and it is to be regarded as due to the same agency.

The instance of intrauterine inoculation affords the best example of the invasion of the various coats of the uterus. There are not alone great changes, similar to those already described, to be made out in the mucous membrane and decidua tissue, but the muscle of the uterus is *œdematosus* and infiltrated both with large numbers of bacilli and with inflammatory elements. The inflammation extends to the serous surface, which is covered by a mixture of fibrin and cells, among which large numbers of bacilli occur. The intervillous spaces con-

tain great numbers of bacteria, and the surface of the mucous membrane is covered with a rich growth of the organisms, among which very few tissue or emigrated cells can be made out.

The increased susceptibility of the pregnant compared with the non-pregnant uterus to the localization, development, and injurious effects of this organism, is very conclusively illustrated by this series of cases, and indeed as much so by the study of the relation of the bacilli to the various portions of the affected uteruses as by the observation of the differences in behaviour of the pregnant organ on the one hand and the non-pregnant on the other. From the pathological process observed in the affected structures produced by the intravenous and the intrauterine inoculations, and from their agreement with the observed conditions in the spontaneous case, it could be easily imagined that the presence of the bacilli in the uterine cavity might have brought about a premature labour in the first case, as it demonstrably did in the experimental ones. In none of the experimental cases of uterine infection were localized inflammations met with similar to those in the pleura and pericardium of the spontaneous case.

The Remaining Organs.—Very little need be added concerning these. With the exception of the liver, which in many cases showed focal areas of necrosis of the liver substance, focal lesions were not found in the viscera, in which no localization of the bacilli took place. The hepatic necroses, it must be assumed, were produced not by the bacilli themselves, but by the soluble products of their growth. However, until a certain and convenient method for the cultivation of the organisms is found, this point can not be entirely settled, for we know that the use of cover-slip preparations for determining the presence or absence of micro-organisms in tissues is not in itself conclusive. We have, however, abundant analogies tending to show that similar lesions can be produced by the products of bacteria, and it is therefore, perhaps, not assuming too much to consider that this one also is capable of manufacturing a toxic substance which, in the absence of organisms themselves, may be destructive to tissue elements situated at a distance from the seat of its formation.

RÉSUMÉ AND GENERAL CONSIDERATIONS.

The organism described in the foregoing pages must, we think, be regarded as belonging to the pleomorphic bacteria, although it may not be entirely clear precisely with which group it should be classed. Assuming that we have before us the entire series of its developmental phenomena—which, in the absence of a knowledge of more durable forms, or the conditions under which it may vegetate outside of the animal body, may well be questioned—it is possible to exclude it from certain groups of more highly organized bacteria. The complete absence of branching, both apparent and real, removes it from the group of *cladothrix* on the one hand and *streptothrix* on the other. With the *leptothrix* forms it has more in common ; and from its tendency to grow into long and slender threads, and in cultures to appear in segmented filaments, it might, provisionally at least, be classed with this group. A further support for such a classification might be found in the presence of a sheathlike mantle, in which the chromatic particles are distributed (*crenothrix*), were it not for the fact that the nature of these particles hardly permits of their being regarded as independent bacterial cells ; nor do they ever appear separately and independently of the sheath in preparations obtained directly from the animal body, while in the cultures no suggestion of a sheath is to be made out.

Notwithstanding the great variations in size, and the slighter differences in staining properties which were observed in the organism as obtained from the original source, and again noted in all subsequent cases, there can be no reasonable doubt that a single species existed in the infected animals as well as in the cultures. It would be, to say the least, remarkable if, in the various transplantations from animal to animal, and from these to culture, and then back again to animals, two distinct organisms had remained together in so nearly the same ratio as in this instance. It is no uncommon experience to have under observation mixtures of bacteria in cultures, but it usually happens that one or the other kind possesses the power of greater and more rapid growth, so that after a series of trans-

plantations the weaker or more feebly growing organism is overgrown, and, if not entirely destroyed, so greatly reduced in number that especially favourable conditions are required in order that it may again make its appearance. What happens in artificial cultures takes place more rapidly and certainly when the living animal body becomes the territory upon which the striving micro-organisms seek to implant themselves. We know, from the results of the earlier experiments with mixtures of bacteria carried out by Koch, that the animal body is capable of separating pathogenic species of bacteria from non-pathogenic, as well as different pathogenic forms from one another. It is therefore hardly to be conceived that two or more distinct species should have continued to flourish together side by side through so long a time, and under such varying conditions as were present in these experiments. On the other hand, it is no new fact in bacteriology to find a single species of bacteria under such manifold forms as in the present case. Indeed, it may, after all, be questioned whether the variations between the long and the short forms and the dotted and the solid rods are as great as between the extremes of *proteus vulgaris*.

The greatest changes observed were in the artificial cultures, for in the last removes the irregularity of staining quite disappeared, the bacilli appearing now as solid rods, many of which could be seen to have divided by transverse segmentation. Even if the evidence were not at hand that, as obtained directly from the animal body, a solid form is occasionally observed among the irregularly staining bacilli, and that the inoculations into rabbits of the artificial cultures containing solid forms cause a typical pathological process with a return of the characteristic markings in the micro-organisms, we have abundant observations upon the changes in the morphology of the common bacteria that may be produced by differences in the composition of the media upon which they are grown, and upon such of their physiological functions as the production of pigment, of acid or of alkali, of toxic substances and of spores.

The peculiar selection which this organism exhibited in respect to the substances upon which it was finally brought to develop out-

side the body of infected animals need excite no especial surprise if it is remembered that not a few widely distributed and quite commonplace bacteria—namely, the gonococcus, the micrococcus lanceolatus, and the streptococcus pyogenes (Marmorek)*—show at times similar preferences for certain media, and refuse entirely to grow upon the more ordinary artificial nutrient compounds. Moreover, as is well known, there are not a few bacteria which, while easily demonstrable in cover-slip preparations, have not been successfully cultivated at all. Whether the mouth bacteria, which are so refractory in this respect, might not be brought to regular development upon media that more nearly represent the fluids and tissues of the animal body than do our artificial compounds, may well be asked. Thus the behaviour of the more purely parasitic bacteria upon the ordinary culture media has made it quite unmistakable that under the most favourable circumstances we have in the latter only doubtful substitutes for the tissues themselves, a fact which these results confirm and extend. The further demonstration of differences in the individual organs in their behaviour toward the growth of this bacillus is also of interest in connection with the metabolism of these organs and the observed localizations of the micro-organism within the body.

The question of the effects exerted upon bacteria by the soluble constituents of the different tissues has recently been studied in Schottelius's laboratory, and several publications have already appeared upon this subject. The organs employed were the thyreoid gland (Kopp),[†] the pancreas (Kotlar),[‡] the kidney (Henssen),[#] and the liver (Onufrowicz).^{||} Culture media prepared from cold and filtered infusions of these organs affected the growth of bacteria in various ways. Some were restrained, others accelerated, and some suffered changes in their morphology. The last were not of a permanent character, a reverse change taking place upon retransplantation to the ordinary nutrient materials.

* *Annales de l'Institut Pasteur*, No. 7, 1895.

† *Centralblatt f. Bakteriologie u. Parasitenkunde*, Band xvii, 1895, p. 81.

Die Lebennährböden und ihre Beziehung zum Wachsthum und Differenzierung der Bakterien. Inaugural Dissertation, Freiburg, 1894.

The study of Henssen in particular seemed to indicate that the *staphylococcus pyogenes aureus*, which in rabbits often produces abscesses in the kidney, grows very slightly upon infusions of this organ. Bearing upon this point are our studies upon the relative values of the different solid organs, as culture media and their relation to the localization of the bacilli within the body. It was, however, not possible to make any comparison of the behaviour of the organs and of media prepared from them, as upon the latter no indication of growth was ever obtained. The two organs upon which a growth was never obtained were the liver and the spleen. The kidney permitted the transplanted bacilli to develop, yet the growth was slight in amount and more quickly refused further transplantation than that present upon the other organs. These facts are not altogether without significance when we compare them with the observed localizations of the bacilli within the organs. The kidney and spleen in no instance showed focal lesions produced by the bacilli themselves, and when such were found in the liver this organ was invariably the seat of previous disease. On the other hand, the growth upon the removed organs was more vigorous in the case of the pleura, the lung, the pericardium, and the uterus, and these were the situations in which the bacilli produced the greatest damage within the body. It is an attractive idea to consider that there may be a relation between the predilection shown by micro-organisms for certain viscera and certain chemical substances contained within these structures. According to this idea, the foetus of the rabbit would contain these substances in larger quantities than the viscera, or in a more easily assimilable form. These hypothetical substances also would appear not to pass over into the fluid in which the organs are macerated. The foregoing is to be considered at most as a suggestion for which the only basis are the observed facts enumerated. It is true that a contrary mode of explaining the facts is not to be excluded—namely, that in some of the organs substances actually injurious and inhibiting in their nature may be contained, to which the variation in or entire absence of development is due. It is not possible to explain the distribution of the bacteria solely through

the peculiarities of the blood supply in the organs. To make this clear it need only be asked why the kidney, in which the arteries are terminal, should always escape, and the voluntary muscles sometimes be the seat of abscess development?

The probable nature of the deeply staining dots, globules, and cylinders within the bacilli is of interest. That they contain the main if not the entire chromatin material is already evident from the descriptions, as well as from the photographs and drawings. They present, however, certain peculiarities which are worth mentioning. The bacilli are easily stained in the basic anilines, gentian violet, fuchsin, and methylene blue, but least rapidly in the last dye. In stained specimens which have been washed simply in water the threads stain fairly, although not quite uniformly; however, the deeper-stained dots can still be observed within the deeply tinted protoplasm. The use of a weak decolourizing agent (1 to 1,000 acetic acid) brings about a sharp differentiation between the chromatic particles and the matrix of protoplasm in which they lie. The use of stronger decolourizing solutions—as, for example, one-per-cent sulphuric acid, as is used in Neisser's method—causes instantaneous and complete decolourization, and attempts to obtain double staining in methylene blue and Bismarck brown by Ernst's method were entirely unsuccessful. On the other hand, immersion in moderately strong solutions of Bismarck brown for some minutes produced the effect obtained only with the use of bleaching agents after staining in fuchsin or gentian violet. The sharpness and distinctness with which the rods stained in haematoxylin were remarkable. This reagent stained the chromatic particles a deep blackish purple and the protoplasmic envelope a faint violet, much resembling the behaviour of the nuclei and protoplasm of tissue cells in this respect. So far as this reaction can be trusted, the sharp differentiation which exists between the matrix or thread and the chromatic particles might be considered a division into nuclear and protoplasmic constituents, especially as in unstained specimens the chromatic particles can be seen and possess a different refraction from the matrix. Moreover, the occasional solid forms take a tolerably uniform haematoxylin stain,

indicating—as, indeed, the aniline colours prove—a uniform diffusion of the chromatic material.

From the observed facts of the behaviour of the stained particles, they would appear to differ from the “sporogenous bodies” of Ernst* and Neisser,† although, perhaps, agreeing better with the “metachromatic bodies” of Babes,‡ such as have been described in a number of micro-organisms and occur regularly in the xerosis group of bacteria. It may be questioned whether in our bacillus these bodies are not different from those which they studied, as there is certainly great difference in respect to the resistance which they offer to staining agents. Now, since Bunge# has shown that the “sporogenous bodies” of Ernst have no relation to spore formation, it would seem hardly necessary to mention them, and we should not do so except to repeat in this place that, notwithstanding the possession of many chromatic particles, our experiments make it very probable that the bacillus pyogenes filiformis does not produce spores at all; certainly nothing resembling these structures was ever encountered in cover slips from animals or those prepared from cultures.

Evidences of degeneration, such as vacuolization of the bacilli, were occasionally observed in specimens obtained directly from the animal. In slightly growing artificial cultures, and especially in such as had attained their development upon organs and remained a few days at the room temperature, they were very common. The degeneration of the characteristically dotted forms was somewhat different, consisting of a gradual fading of the whole organism until it was seen with great difficulty even in deeply stained specimens. The chromatic particles became altered in outline, irregularly notched or worm-eaten in appearance, and fragmented, often only mere specks remaining to indicate their former presence. It was noticed, and it was certainly a curious phenomenon, that from the degenerated forms a new growth had taken place in the cultures—

* *Zeitschrift f. Hygiene*, Band v, p. 428.

† *Ibid.*, Band iv, p. 165.

‡ *Zeitschrift f. Hygiene u. Infektionskrankheiten*, Band xx, p. 412.

Fortschritte d. Medicin, Band xiii, 1895, iv, 20 u. 21.

that is, a characteristic solid outgrowth from such a feebly staining rod was occasionally seen. This indication of the change of morphology in the first generation was unmistakable, and also served to prove that the bacilli in the cultures, notwithstanding the difference in staining properties, were the offspring of the transplanted germs.

Regarding the behaviour of the organism in the tissues a few facts should be emphasized. The bacillus is capable of producing in rabbits all forms of infections, from serous inflammations and abscess formations to extensive necrotizing inflammation and septicæmia. The lesions which it produces varies with the mode of inoculation and the place of localization. In the pleura, pericardium and peritonæum it causes serous and fibrino-serous inflammation; in the cerebral membranes, purulent infiltrations; in the viscera, abscesses and cell necrosis; in the subcutaneous tissues and muscles, extensive necrosis and inflammation. The fibrin formation is not great, notwithstanding the remarkably thick and shaggy character of the pleural and pericardial exudates. Nothing could be more suggestive of a fibrinous inflammation than are these appearances, and yet the histological examination shows that there is a small amount only of actual fibrin (staining in Weigert's stain) and that the shaggy appearance is brought about by the great numbers of felted bacilli which form a false membrane upon the serous membranes in the meshes of which the pus cells are contained. Various kinds of necrosis are produced. Simple hyaline cell death, as in the liver; more extensive hyaline necrosis, as in the voluntary muscles; great fragmentation of cell nuclei, as in the intermuscular septa, subcutaneous tissues, and in localized areas in the viscera; and large, spreading, necrotic foci, much resembling the caseation of tubercle, as in the lungs and brain.

The bacilli show in the lung a marked tendency to invade the bronchi. From the descriptions of the pathological process in this situation it will be recalled that these were greatly affected. The bacilli do not grow nearly so freely in the lung substance as in the bronchi; they give rise to dense masses, often sheaflike in form, which project into their lumina and are covered with an exudate

which may fill the tubes, and in which they again spread. From this fact it may be inferred that the organisms are strongly aerobic in character; indeed, if there were any differences observed in rapidity and extent of growth in the artificial cultures they were in favour of the aerobic tubes.

The endarterial changes which are so common in the lungs and are less frequently met with in the skin, heart, muscle, and brain, are of interest as indicating the short space of time required for their development, the manner in which the new growth appears, and the relation borne by the altered vessels to the pathological process in the viscera. Where there is a direct extension of the destructive action of the organisms to the vessel wall, the damage done the latter is usually great, and the result is not a proliferation of cells in the intima, but actual necrosis of the vessel and thrombosis; on the other hand, when the vessels lie within the affected area or near to a focus of bacillary invasion and are themselves not invaded by the organisms, or exposed to the action of so intense a poison as to cause death of a part of the wall *en masse*, then the proliferative changes are to be observed. In view of the other evidences that have been adduced to show that a part of the action of this organism is attributable to a toxic substance which it forms, it is not difficult to see in the endarterial changes mentioned the operation of the same cause. We think that we have brought evidence in the location of the karyokinetic figures of the participation in this process of proliferation of the most superficially placed intimal cells, perhaps even of the endothelium itself. We were distinctly less successful than Hektoen * in demonstrating a constant layer of flattened endothelial cells upon the surface of the new growth; and, indeed, we even doubt the conclusiveness of this observation as demonstrating the non-participation of the endothelium in the multiplication of the cells. It is conceivable that the shape of the superficial cells might be accounted for in no small degree by the blood pressure to which they were subjected. However, it is not our intention to give a critical review on this sub-

* *The Journal of Experimental Medicine*, vol. i, No. 1, p. 112.

ject, as it has just been done in an excellent manner by Hektoen, to whose paper we would refer.

The observation of the occurrence of great numbers of large mononuclear elements within the lumina of the blood vessels affected by the endarterial changes brings up the question of the function of the vascular endothelium as well as the question of the extent of its participation in the inflammatory phenomena. That the vascular endothelium possesses the power of engulfing particles—micro-organisms and others—the studies of Ponfick,* Neisser,† Touton,‡ Met-schnikoff,[#] Wyssokowitch,^{||} and Monti[△] have shown. And it has also been rendered probable, through the observations of Neisser and Touton, that when filled with bacilli the endothelial cells may become detached from the vessel wall and be found free in the blood current. Our observation of mitosis in occasional large mononuclear cells within the lumen of the affected vessels lying among other mononuclear and polymorpho-nuclear elements with resting nuclei, makes it not improbable that under certain pathological conditions these cells may be the source of some of the inflammatory elements, perhaps, indeed, of the so-called macrophages.

In passing, it may be well to refer again to another unusual phenomenon which was observed in the lung—namely, the invasion of the cartilages of the bronchi by the bacilli, while the inflammatory cells were rarely seen in this structure. It is also of interest to note that in the cases of pericarditis, although there were no muscular changes in the depth of the heart, yet circumscribed patches of interstitial myocarditis were not infrequently met with in this situation.

It seems probable that in the growth of the bacilli in the tissues of the rabbit a certain order was preserved. The tendency to grow in parallel lines, to form sheaflike masses with a wide base and an acute apex, and frequently to surround in a radial manner a central

* Virchow's *Archiv.*, 1869, Band xlviii, p. 1.

† *Ibid.* 1881, Band lxxxiv, p. 514.

‡ *Ibid.* 1886, Band civ, p. 381.

* Pathologie comparée de l'Inflammation, p. 167.

|| *Zeitschrift f. Hygiene*, 1886, Band 1, p. 3.

△ *Bullettino della Società Medico-Chirurgica di Pavia*, 1895.

group of cells, was too striking a peculiarity to be passed by. The sheaflike development observed upon the pleura and in the lung, but especially in the uterine glands, and the radiate arrangement in the brain abscesses, occurred with too great frequency to be neglected. Whether this feature will be found to be so constant as to serve for the distinction of this organism in the same manner as the ray fungus is distinguished can only be conjectured.*

This organism must be classed with the bacteria which produce gas within the tissues of the living animal. Unfortunately, attention was not directed to this point early enough in the course of the experiments to observe whether or not it produced gas in the serous cavities. Gas bubbles occurred, however, in the inflamed subcutaneous and mediastinal tissues and in the brain. The relation of the bacilli to the gas cavities was variable. In the brain the margin of the cavity contained a large number of the bacilli; in the subcutaneous tissue the collections of gas might be independent of special accumulations of the organism. The most interesting feature was the production of gas in the closed cavity of the uterus, both in the original and in the experimental cases. Here the walls of certain segments were greatly distended, attenuated, and translucent, and the anatomical structures greatly altered by the pressure to which they had been subjected. Bacilli occurred freely within these segments of the uterus.

The effect of local conditions in determining the localization and development of the bacilli is best shown by the consideration of the behaviour of the pregnant as compared with the non-pregnant uterus. If, in the differences observed, modified circulation can be excluded from playing a definite part, we have then to consider that the new anatomical and physiological processes introduced by the pregnancy are responsible for them. It is suggestive, to say the least, to view this fact in the light of the favourable medium which the rabbit's foetus, as compared to the uterus, afforded for the growth of the micro-organisms.

* It is interesting to note that the cladothrix (see below) which Eppinger obtained from a case of pseudo-tuberculosis and brain abscess in a human being showed a similar palisade-like form of growth in the tissues.

Although leucocytes were abundantly attracted to the inflamed parts in the rabbit, the inclusion of bacilli by cells was an unusual phenomenon. In guinea-pigs, which proved to be distinctly less susceptible to the organism, phagocytosis occurred to a not inconsiderable extent. We have already drawn attention to what we have denominated pseudo-phagocytosis, by which is meant the invasion of dead cells by bacteria, producing appearances which might be mistaken for phagocytosis. This occurred in sterile organs removed from the body which had been used for growing the bacilli.

Other laboratory animals than those noted did not prove susceptible to inoculations with even large quantities of infectious material containing this organism. Thus, in the mouse both subcutaneous and intrapleural inoculations, in the pigeon subcutaneous, intramuscular, and intraperitoneal injections, and in the dog intravenous inoculations, produced no noticeable effects.

The pleomorphic micro-organism described in the foregoing pages has not, so far as we have been able to examine the literature, been described before, nor has it been met with again by us in the year which has elapsed since its first detection. It remains, therefore, to be seen whether its occurrence is so exceptional as these facts would indicate. However, it is not improbable that it has been seen in cover-slip preparations by others who, owing to its refractoriness in this respect, may have failed to cultivate it. The name which we propose for it is intended to cover, in the first place, its most striking morphological feature, and, in the next, its chief pathological effects.

REVIEW OF THE LITERATURE.

If we except the micro-organism which is believed to cause the different forms of actinomycosis, the organism of madura-foot disease, and the various varieties of proteus, the number of pleomorphic and more highly developed bacteria which have been found associated with pathological processes in human beings and animals is reduced almost to nil, notwithstanding that complex bacterial forms, such as *cladothrix*, *crenothrix*, *streptothrix*, etc., are widely distributed or-

ganisms in Nature. These have, so far as is known at the present time, played a very inconsiderable part in the causation of pathological conditions, either in man or in the higher animals. It is this fact which lends to the present organism a greater and perhaps more general interest than it otherwise would command. If we exclude diseases produced by the actinomyces organism, the proteus bacilli and the micro-organism of madura-foot disease, and, furthermore, disregard, for the moment, the fact that the bacillus tuberculosis and the bacillus diphtheriae * have been shown sometimes to possess a more complex structure than had hitherto been ascribed to them, we can quickly review the literature of the remaining pathological processes associated with the pleomorphic and more highly organized bacillary forms.

Rosenbach,† in 1887, drew attention to the relation which existed between erysipeloid, or, as it is also known, erysipelas chronicum and erythema migrans and a cladothrix form of bacterium. The infectious agent, according to him, is found in animal substances which are undergoing decomposition, thus exposing to infection with it such persons as are employed in handling partly decomposed articles. The disease exists among cooks, dealers in game, butchers, tanners, fish handlers, oyster shuckers, and finally dealers who trade in cheese, herring, etc. As the infected material comes in contact almost exclusively with the hands, the affection is found especially upon the fingers, and, as might be expected, other parts of the skin may become infected secondarily from this source. Extending from the point of inoculation there is a deep-red, often livid swelling, with a sharp periphery much resembling the erysipelatous rash. The general bodily health and the temperature are not affected. The disease does not run a definite course and generally comes to a spontaneous end in one, two, or three weeks. Rosenbach obtained from cultures taken from the affected skin a micro-organism which he identified as a form of cladothrix. He found that it grew best upon gelatin at a low temperature, and was able to produce by the inoculation of

* Fischel, Coppen-Jones, Bruns, C. Fraenkel.

† *Archiv f. klinische Chirurgie*, 1887, p. 346.

his own arm with such a culture a condition similar to the natural erysipeloid.

Naunyn* found a peculiar vegetable micro-organism in the haemorrhagic infiltrated cerebral membranes and upon the endocardial excrescences in a case of chorea. The organism consisted of threads which possessed a brown colour, owing to impregnation with salts of iron. It was regarded by Neumann and Baumgarten as belonging to the threadlike fungi, and by Zopf as occupying an intermediate position between the *cladothrix* and *leptothrix* forms of micro-organisms. It must be admitted that this case is not perfectly clear in its interpretation, particularly as Baumgarten and Neumann have drawn attention to the fact that at the time of the isolation of this organism the water-supply which had been used for washing off the organs from the case contained large numbers of *leptothrix* and *cladothrix* forms which resembled in appearance those which Naunyn found in the infiltrated cerebral membranes and upon the heart valves.

A number of publications upon the aetiology of pharyngomycosis *leptothrica* (Chiari) have appeared, from which it is rendered probable that the condition first described by B. Fraenkel under the name of *mycosis tonsillaris* (*pharyngomycosis*) *benigna* is due to the invasion of the affected structures of the throat by a *leptothrix* bacterial form, possibly the *leptothrix buccalis*.

Rabe † described a *cladothrix* which he obtained from purulent phlegmons in the dog. He observed in two dogs a diffuse inflammation of the skin and subcutaneous tissues of one of the fore paws which underwent partial necrosis in the first, and gave rise to suppuration of the glandulae cervicales superficiales in the other. The latter abscesses were surrounded by granulation tissue of rather peculiar appearance. A third animal, which about three months previously had had an abscess of the parotid gland, died four days after a subsequent illness of an acute peritonitis. In the last animal, as well as

* *Mittheil. a. d. med. Klinik zu Königsberg in Pr.*, 1888, p. 296. (Ref., Baumgarten's *Jahresbericht*, Band iv.)

† *Berl. thieraerzl. Wochenschrift*, 1888, p. 65. (Ref., Baumgarten's *Jahresbericht*, Band iv.)

in the two previous ones, Rabe found in the pus many grapelike, lobulated, pale granules, which upon magnification resembled the actinomyces organism. Cultivation experiments failed, but the inoculation of a goat and of rabbits gave, according to Rabe, positive results. It was possible, by the use of infected material, to produce local abscesses which, however, usually healed, but in one instance caused the death of the experimental animal in six days. In the last case only was the organism recovered from the site of inoculation.

Schmorl* has described a pathogenic, threadlike bacterium (*streptothrix (?) cuniculi*) which he obtained from the rabbit. He found it in an infectious disease of these animals which began in a rapidly spreading necrotic inflammation of the lips and subcutaneous tissues, and which give rise later to inflammations of the serous membranes (pleura, pericardium, and peritonæum) and also of the lungs. He regarded the organism as belonging either to the group of leptothrix or cladothrix. He succeeded in obtaining it in pure culture upon blood serum where it grew only under anaerobic conditions. Inoculated upon rabbits, it gave rise to the same pathological conditions as in the cases of spontaneous infection. In addition to rabbits, the only other laboratory animals which were susceptible were mice; guinea-pigs, dogs, cats, pigeons and hens having shown themselves refractory to the inoculations. Schmorl concludes that in the bodies of human beings and of the guinea-pig this micro-organism will grow only if it is associated with pus-producing bacteria which prepare for it favourable conditions of growth; alone, it is pathogenic neither for man nor for guinea-pigs.

Eppinger,† in 1890, made the interesting observation that cladothrix forms other than actinomyces might be pathogenic for human beings. He reported the case of a man fifty-two years old in whom he found at autopsy a chronic brain abscess which had perforated into the right lateral ventricle and had given rise, in addition, to a purulent cerebro-spinal meningitis. In the pus and in the walls of the abscess, as well as in the miliary purulent foci in the adjacent

* *Zeitschrift f. Thier medicin*, 1891, p. 375. (Ref., Baumgarten's *Jahresbericht*, Band vii.)

† Zeigler's *Beiträge zur pathologischen Anatomie*, Band ix, p. 287.

brain substance, many threadlike and apparently branching organisms arranged in groups and in bundles occurred. Lesions resembling tubercles which were in part calcified and in part fibroid existed in the lungs. The bronchial glands were enlarged and calcified, and the supraclavicular glands of the right side were in the same condition. Although this peculiar organism could not be found in the tubercles in the lung, fragments of the threads were discovered among the detritus of the bronchial as well as of the supraclavicular glands. Eppinger cultivated the *cladothrix* from the pus of the brain abscess upon various culture media. It grew best upon sugar agar and upon blood serum; upon gelatin it grew faintly, but upon potato and in bouillon the growth was regarded as quite characteristic. All the cultures gave the same result upon microscopical examination, and showed the presence of apparently branching threads of various lengths, the apical ones of which resembled cocci. Long threads without branches and smaller bacterialike, cuboidal cells occurred among the branching forms. The first possessed a gliding, and the cubical cells a rapidly whirling or rotary movement. Eppinger observed, in hanging drops containing the small cubical cells, a development of the apparently branching threadlike forms. Experiments upon guinea-pigs and rabbits led to the production of widespread pseudo-tuberculous nodules in which the same organism was again found, and from which it could be obtained in pure culture. Owing to the peculiar starlike manner of its growth, which in tissues much resembles the arrangement observed in the brain abscesses of our experimental animals, the name of *cladothrix asteroides* has been proposed for the organism by its discoverer.

This brief review comprises the cases in the literature, so far as they have come to our notice, in which pathogenic effects have been observed in man and animals, with the exceptions mentioned, through the action of the pleomorphic and highly organized bacteria. The list is still small; and of this number not all have fulfilled the requirements for admission to the ranks of the recognised pathogenic micro-organisms. It is considered that the proof is con-

70 *The Bacillus Pyogenes Filiformis and its Pathogenic Action*
clusive of the pathogenic character of the organism described in
these pages.

In conclusion, I wish to express my indebtedness to Dr. Welch
for many valuable suggestions during the progress of this study, and
to thank Dr. Hoen for the photomicrographs which he kindly made.

EXPLANATION OF PLATES.

PLATE IX.

Fig. 1.—Photomicrograph of bacilli derived from the exudate within the uterus of the spontaneous case. Gentian-violet and acetic-acid method of staining. Leitz, one twelfth in. homogeneous immersion objective.

Fig. 2.—Photomicrograph made under the same conditions as the preceding of a cover-slip preparation obtained from a culture upon the foetus of the rabbit.

PLATE X.

Fig. 3.—Experimental abscess of the brain of the rabbit, showing the central mass of pus cells and the peripheral zone of bacilli. Leitz objective No. 3, ocular No. 3. Gentian-violet staining.

Fig. 4.—Edge of the previous specimen under the magnification given by Leitz, one twelfth in. homogeneous immersion objective, ocular No. 3.

Fig. 5 shows the sheaves of bacilli in the glands of the uterine mucosa and single bacilli in the stroma of the mucous membrane and in the muscle. Leitz objective No. 6, ocular No. 3.



FIG. 1.



FIG. 2.

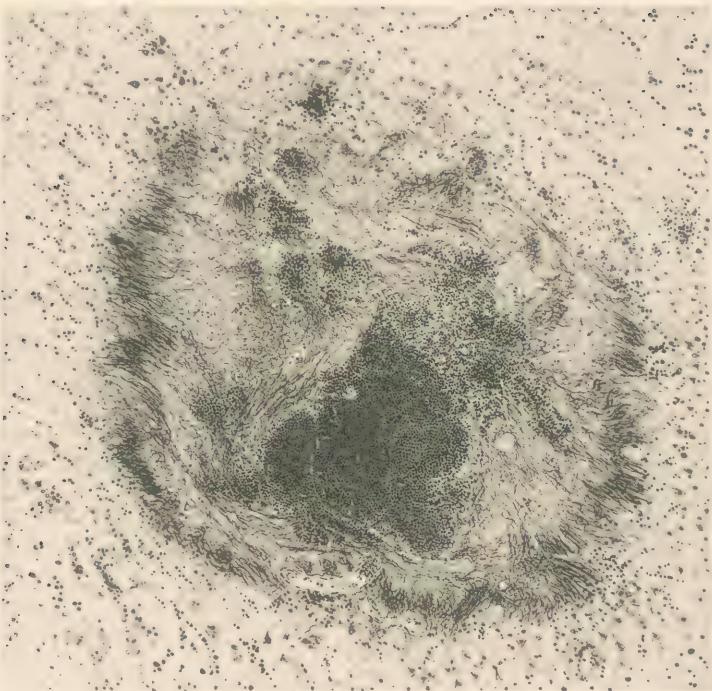


FIG. 3.



FIG. 4.



FIG. 5.

